FACIAL NERVE REPAIR:

A comparison of the anastomotic methods used to repair a divided facial nerve in the rat.

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DECLARATION

I hereby declare that this thesis has been composed by me alone and all the work is entirely my own unless otherwise stated.

ABSTRACT OF THESIS

The facial nerve is often damaged by a malignant tumour of the parotid gland. There is no accepted best method of anastomotic repair. This study uses the buccal division of the rat facial nerve as the experimental model to compare the use of absorbable sutures, non-absorbable sutures, fibrin glue and collagen tubes in the repair of the divided nerve. The rat sciatic nerve was used as the experimental model to compare epineurial and fascicular repair of a multi-fasciculated peripheral nerve.

All the experiments were randomised, blind, controlled trials and all the surgery was performed by the author. The results were assessed photographically, electrophysiologically and histologically. No one material was found to produce a better anastomotic result over any other material and the epineurial repair had similar results to the perineurial repair.

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1. FACIAL NERVE DAMAGE

1.1 INTRODUCTION

The facial nerve supplies humans with the ability to visually express emotion. This is of the highest importance for every human being and it is only too easy to understand that striking disfigurement has captured the interest not only of doctors but artists as well. The famous sculpture from the 'Marten's' group at Frauenhaus in Strasbourg 'the man with the facial palsy', created by the Dutch master Gerhaert van Leyden in the 15th century, is an attempt to portray suffering. Conversely Leonardo de Vinci's Mona Lisa, at present hung in The Louvre, Paris, demonstrates a haunting half smile which might well be the result of a mild bilateral facial palsy (Adour, 1987).

Facial paralysis was recognised by ancient peoples. Sushruta (1963) called it 'Arditam'. "Pregnant women, mothers immediately after parturition, infants, old and enfeebled persons are most prone to fall victim to this disease. It has also been known to result from excessive haemorrhage or loss of blood. The local VAYU, extremely enraged or aggravated by continuous talking in an extremely loud voice, chewing of hard substances, loud laughter, yawning, carrying extremely heavy loads and lying down in a uneven position on the ground, finds lodgement in the regions of the head, nose, upper lip, chin, forehead and the joints (inner cornea) of the eye and produces

the disease called Arditam by distorting the face".

Historical

The Greeks in Alexandrian schools were among the first to attach any importance to the brain. Alcmaeon of Crotona (c 500 BC), an accomplished anatomist and embryologist, attributed man's higher functions to the brain's activities in contrast to the then widely held view that it influenced mood by secreting phlegm. Erisastratus of Chios (c 300 BC) described the separate existence of sensory and motor nerves and speculated on their communication with the system of ventricles in the brain. He thought the ventricles were the source of pneuma or 'animal spirits' which flowed down the hollow nerves to activate muscles which is the first description of axonal transport (Ribchester, 1986).

Paul of Aegina (625 - 690) was the first to postulate possible repair of divided nerves. Avicenna (980 - 1037) further suggested bringing the 2 ends of a severed nerve together and suturing the epineurial tissue (ie, an indirect nerve suture). Da Saliceto (1210 - 1280) and his pupil Lanfranchi distinguished stab injuries, longitudinal and transverse injuries of nerves and suggested different methods of treatment for both. The first successful nerve suture, using the principles first propounded by Avicenna, are ascribed to the School of Bologna. Lanfranchi and De Chauliac (1300 - 1368) popularised nerve suture in France and Switzerland. Ferrara, in the second half

of the 16th century, was the first surgeon to perform a direct nerve suture in which he used a tendon of turtles, soaked in red wine.

Descartes (1596 - 1650) propounded the theory that the brain and spinal cord developed by the flow of animal spirits out of the heart. He also described nerve fibres as channels through which information (in the form of animal spirits) was passed between the brain and the periphery. He also suggested that sensory stimulation might lead anatomically to motor function, ie a reflex.

Von Haller (1708 - 1777) first demonstrated experimentally the mode of control exercised by nerves over muscular contractions. He demolished the old illusion that tendons transmitted sensation.

The experimental work of Galvani and Volta during the mid 18th century, established the theory of electrical nervous impulses. Cruikshank (1795) proved that an injured nerve was capable of regeneration and provided a powerful stimulus for the further development of successful peripheral nerve suture.

By the middle of the 19th century the concept of the cell as a unit structure, from which complex tissues were constructed, became generally accepted mainly as a result of the work of Schwann, Schleiden, Purkinje, Valentin, Henle and Virchow.

Improvements in microscope lens technology and important developments in histological fixation and cutting techniques, were crucial factors. Remak first described the myelin sheath (despite the renaming later after Schwann), pioneered the study of the neural tube and described the 3 germ layers of vertebrate enlages, ie the ectoderm, mesoderm and endoderm. Pupils of Remak, His and Kolliker, further contributed to the theory that neurites are formed by outgrowth of cellular processes from the cell body (Ribchester, 1986).

Charles Bell is widely recognised as the father of the facial nerve. He had a deep artistic feeling and in 1806 published the work 'Essays on the anatomy of expression in painting'. Homer had already highlighted the link between the facial muscles of various animals and the range of passions and moods. The human is quite superior to all other animals in his ability to communicate emotion from facial expression. In a paper to the Royal Society in 1821 entitled 'On the nerves, giving an account of some experiments on their structure and functions which lead to a new arrangement of the system', Bell described experiments involving the sectioning of the facial nerve and the trigeminal nerves of the ass. When the facial nerve was divided, facial movement ceased but there was no sign of pain or change in sensibility. The slightest touch of the exposed facial nerve produced muscle convulsion but no pain. The facial nerve was, therefore, obviously not only the nerve of respiration but also the general nerve of expression. Sectioning of the trigeminal

nerve diminished sensation to the area supplied and stimulation of the exposed nerve, produced intense pain. Bilateral division of the trigeminal nerve prevented the animal chewing. He published 2 works over the ensuing 9 years illustrating his changing ideas in neurology of the face which definitely separate the functions of the trigeminal and VII nerves.

The work in 1824 was entitled 'An exposition of the natural system of the nerves of the human body'. In 1830 he published 'The nervous system of the human body'. One cannot improve on Bell's classic description of facial paralysis 'The muscles of the cheek on the left side are wasted and there appears to remain nothing but the thin integument which hangs up on the side of the face, as if dead, without having any action in them or wrinkles, as in the right cheek, and when he speaks his cheek is alternately puffed out and then collapsed. The air first distending it as if it were a bag and then escaping at the angles of the mouth. The whole mouth is drawn to the right side, thus producing the most remarkable distortion of the face. Whatever action there is in the mouth is altogether owing to the contribution of the muscles on the right side of it and the left angle hangs loose and it is quite passive, and the saliva is allowed to flow constantly out of the lower lip on this side'.

In 1829 he described the sign eponymously named after him. In a patient who had suffered a traumatic facial paralysis, probably from an injury to the nerve near the stylomastoid foramen, he

noted the eyeball rolled upwards when the eyelids were closed. This is a normal phenomenon with closing the eyes but it is not normally seen unless the orbicularis oculi is paralysed (Miehlke, 1973).

Duchenne (1872) further elucidated the mechanism of facial expression with observations on electrical stimulation of the muscles.

Brissaud (1895), later in the 19th century, described the double innervation of the face based on the disassociation between voluntary and mimetic expression in pseudobulbar palsy, compared with the masked facies of Parkinsonisms.

Flatau (1897) then fully described the normal and pathological anatomy of the trigeminal, facial and cochlear nerves. Pitres (1913) was one of the first to describe clinical synkinesis after Bell's palsy.

Philipeaux and Vulpan (1870) first tried to bridge nerve defects by using 3 nerve transplants. They were followed by Le Tievant (1873) and Mayo Robson (1887).

In 1842, Flourens carried out successful nerve anastomosis in birds. In 1879, Drobnik united, in a man, the peripheral end of the facial nerve to the central end of the external branch of the spinal accessory. After several months the features had become more symmetrical. In 1895, Ballance sutured the facial

nerve to the spinal accessory after trauma during a mastoid operation.

The functional result was, unfortunately, poor. A variety of surgeons describe nerve anastomosis in the early 20th century (Ballance and Duel, 1932) but Cushing in 1903 reported a successful facial accessory anastomosis.

The choice of the nerve to anastomose lies between the hypoglossal nerve, the descendens hypoglossi and the spinal accessory. Several reports of successful hypoglossal facial anastomosis began to appear beginning in 1903 which are reviewed by Ballance and Duel (1932). At that stage most of the information regarding the use of the descendens hypoglossi, came from the baboon and rhesus monkey experiments. Ballance (1924) reports favourably on the results of these experiments.

As early as 1895 the glossopharyngeal nerve was suggested as a donor nerve on the grounds that its motor nucleus lies close to the facial nucleus (Ballance and Duel 1932). The severance of the glossopharyngeal nerve causes little morbidity and some early cases had a good result and, indeed, is preferred by some surgeons to the hypoglossal nerve.

Surgery on the intratemporal portion of the VII nerve, includes the decompression operation. Alt (1909) recommended that in cases of acute or chronic otitis, a careful search should be

made for any erosion of the wall of the Fallopian canal and that the canal should be opened and the nerve inspected and freed by carefully wiping away granulations and fragments of cholesteatoma.

Removal of scar tissue within the facial nerve and apposition of divided ends in the canal was reported by Staacke (1903). A similar procedure was carried out by Sydenham (1909) who used a piece of silkworm gut laid between the 2 ends of the nerve and the canal. Function was seen after 3 months. Marsh (1909) had a similar experience with 2 cases, using a bridging piece of catgut between the severed nerves.

Le Tievant (1873) recommended nerve grafting as a possible expedient when the ends of a divided nerve could not be brought together. Ballance and Duel (1932), after multiple animal experiments, reached the decision to employ nerve grafts to join the distal ends of the facial nerve within the Fallopian canal. They highlight the need for good illumination 'The operation cannot be done casually by the light of Heaven', and the surgeon must have high technical ability. They recommended the use of a graft of the nerve to serratus anterior.

Embryology

The facial nerve nucleus lies at first in the floor of the fourth ventricle cephalic to the VI nerve nucleus. The nucleus

then moves caudally and dorsally to the VI nerve nucleus and finally ventrally to take up its eventual position. By the fifth week of intrauterine life, the human embryo has established its branchial arch system. The muscles of facial expression originate from the second (hyoid) arch and the nerve of this arch is the facial nerve. Each arch receives a branch from the nerve of the arch caudal to it, ie the first arch receives the chorda tympani from the second arch and the second arch receives the tympanic branch of the glossopharyngeal (Jacobson's nerve) from the third arch.

Development of the nerve within the parotid gland is poorly understood and controversial. Undoubtedly the nerve is intimately related to the substance of the parotid gland itself. The facial nerve courses through the middle of the parotid gland and for descriptive purposes divides it into superficial and deep lobes but, in practice, the gland is quite continuous from superficial to deep lobes.

1.2 THE PERIPHERAL FACIAL NERVE

Normal Anatomy of Axons

The facial nerve motor nucleus in the pons is the source of 7000 motor axons which innervate the facial muscles (Fisch and Esslen, 1977). Approximately 3000 other fibres join these motor fibres to make up the entire facial nerve peripheral axon population. These latter fibres are composed of:

(a) special visceral afferent fibres to the taste buds of the anterior two thirds of the tongue. These fibres have their unipolar cell bodies located in the geniculate ganglion, with central synapses in the nucleus of tractus solitarius;

(b) general visceral efferent secretomotor fibres to the submandibular and sublingual salivary glands, the lacrimal gland, and mucous glands of the nose and sinuses. These fibres are derived from the superior salivatory nuclei (Diamond and Frew, 1979);

(c) general somatic afferent fibres which are sensory to the skin of the posterior aspect of the external auditory canal and the concha of the external ear. These fibres have cell bodies located in the Gasserian (trigeminal) ganglion.

Responses to injury of these 3 types of fibres (in contrast to the motor fibres of the facial nerve) are not entirely understood. Some investigators feel that secretomotor fibres are more sensitive to pressure lesions and take longer to return to normal function. These fibres tend to be smaller in diameter and less myelinated than the large type A motor fibres emanating from the facial motor nucleus (Waxman, 1980).

The motor facial nucleus lies in the lower third of the pons, deep in the reticular formation. The cells are arranged in small groups to form subnuclei, each of which seems to be responsible for innervating the particular muscle supplied by an individual nerve branch, eg the rostral subnuclei are concerned with innervation of the frontalis and orbicularis oculi, while caudal subnuclei innervate lower face and platysma muscles. The most rostral subnuclei (forehead and upper portions of orbicularis oculi) receive fibres from both cerebral hemispheres, and therefore have crossed and uncrossed supranuclear innervation (Diamond and Frew, 1979).

Anatomy of the Peripheral Facial Nerve in Humans

In the posterior fossa the intrameningeal portion is about 30 mm long. In the internal auditory canal (7 mm long) the nerve is surrounded by a sheath of meninges and is intimately related to

the VIII nerve and nervous intermedius. The nerve now enters the Fallopian canal which is 30 mm long. Initially the nerve runs above the vestibule into the geniculate ganglion where the greater superficial petrosal nerve is given off. It then courses posteriorly at 90° for about 12 mm and curves again 90° inferiorly to the stylomastoid foramen. It emerges from this foramen and crosses the styloid process laterally to reach the parotid gland. It divides into 2 main branches (tempero facial and cervicofacial) which in turn divide into the main terminal branches called temporal, zygomatic, buccal, marginal mandibular and cervical. There are many vertical anastomotic branches which form a nerve plexus. The facial nerve is noted for its anatomical variations. These have been summarised within the temporal bone by Anson et al (1970a) and Baker and Conley (1979) also summarised the many and various anomalies of the course of the extratemporal facial nerve.

The facial nerve has many branches and communications througout its course. The clinically important ones are as follows:

(a) Greater superficial petrosal nerve leaves the geniculate ganglion, courses forward and is joined by the deep petrosal nerve from the sympathetic plexus of the internal carotid. This combined nerve then enters the pterygopalatine fossa as the vidian nerve to end in the pterygopalatine ganglion. It carries taste fibres and secretomotory fibres to the lacrimal gland and nasal mucosal glands.

(b) The nerve to stapedius muscle is given off just distal to the second genu in the intratympanic portion. Stimulation of this nerve by loud noises can be detected by tympanic membrane movement consequent on stapes movement. This forms the basis of an important clinical test.

(c) The chorda tympani nerve arises from the facial nerve at 6 mm above the stylo mastoid foramen and leaves the temporal bone having crossed the middle ear cleft, through the anterior canaliculus which is closely related to the Eustachian tube. It courses forward to join the lingual nerve to eventally reach the submandibular ganglion. It carries taste fibres to the anterior two thirds of the tongue and efferent secretomotory parasympathetic fibres to the ganglion.

There is debate as to whether the facial nerve is spatially orientated in topographic fashion in its extra axial course from the brain stem to the periphery as it is from the cortex to the pontine nucleus. In support of topographic organisation, several authors have presented clinical information which has been shown to be useful for fascicular repair and cable grafting of the nerve at the stylomastoid foramen (May, 1973; Janetta, 1975; Podovinec and Pfaltz, 1976; Millesi, 1977; Kempe, 1980). As opposed to this view several investigators have found that the fibres destined for each peripheral branch are diffusely located in the facial nerve trunk (Sunderland and Cossar, 1953;

Harris, 1968; Sade, 1975; Thomander et al, 1982; Gacek and Radpour, 1982). It is likely that there is some degree of spatial orientation of facial nerve fibres, especially at the level at which the axon processes leave the brain stem nucleus and course towards the periphery (May, 1986a).

Blood Supply

The blood supply of the facial nerve consists of a continuing anastomotic pattern with origin from 3 vessels (Miehlke, 1973). The most proximal portion of the facial nerve (meatal and labyrinthine segments) is primarily supplied by the labyrinthine artery, a branch of the anterior inferior cerebellar artery (Anson et al, 1970). This vessel anastomoses near the geniculate ganglion with a branch of the middle meningeal artery which arises from the internal maxillary artery of the external carotid system.

Distally, blood from this vessel communicates with blood from the stylomastoid artery from the posterior auricular vessel of the external carotid system. This latter vessel supplies the mastoid segment of the nerve and anastomoses with the superficial petrosal branch in the region of the tympanic segment. In addition small branches from the internal carotid system, the caroticotympanic arteries, contribute to the blood supply of the tympanic segment.

Fisch (1979) by injection studies of 22 temporal bones, showed that 2 or even more arteries enter the porus from the cerebellar artery - never from the basilar trunk. They are a little larger than arterioles, in fact 150 - 200 μ. The artery always enters the internal auditory meatus at the anteroinferior region of the porus. The biarterial type runs inferiorly along the bottom of the canal, the monoarterial type - the superior portion of the canal. The artery and veins lie in the epineurium between the periostium of the canal wall and the nerve sheath proper. Anson et al (1970b) have shown that there is an extremely rich anastomotic pattern.

The blood supply to the nerve is good although it has been suggested that an area of relative ischaemia may occur in the labyrinthine portion. Anson et al (1970b) described a middle meningeal artery branching from the internal maxillary artery and the stylomastoid artery branching from the posterior auricular artery supplying the tympanic and mastoid portions. The extratemporal blood supply is also excellent from the occipital, superficial, temporal and transverse facial artery. The suspect area is supplied medially by the anterior inferior cerebellar artery and laterally by the superficial petrosal branch of the middle meningeal artery with the anastomosis at the geniculate ganglion.

The intraneural blood supply is derived from branches that penetrate the nerve sheath and split into small arterioles. These

turn inwards to form a capillary net which opens into a variable number of venules. These are embedded in fibrous septae and run in the centre of the nerve. The intraneural microcirculation may be disturbed in many ways and their function may be affected thereby. If either the extrinsic or intrinsic blood supply is affected by mobilisation or crushing the nerve, the other system must be capable of taking over the supply or function will be compromised. The intraneural circulation is extremely sensitive to tension and elongation but experimental data suggests that peripheral nerves may be mobilised over a considerable length with no or only minimal interference with their microvascular flow (Lundborg, 1970; Bagger-Sjoback et al, 1982). The narrowest part of the Fallopian canal is at its entrance where it averages 0.68 mm in diameter. The facial nerve takes up 83% of the available space of the canal in this segment compared with 73% of the canal in the tympanic portion and 64% in the mastoid area (Fisch and Esslen, 1972).

Normal Nerve Function

Each facial nerve neuron is composed of a cell body (soma, perikaryon), several dendrites which receive afferent input from higher centres in the brain, and one peripheral axon. The sheath of each axon consists of a myelin layer internally, a covering layer of Schwann cell cytoplasm, and a connective tissue layer, the endoneurium, externally. A membrane potential of 90 mV exists across the axon membrane. The total cross-

sectional diameter of these fibres ranges from 2 to 30 . The sheath is deficient or absent at nodes of Ranvier, which are spaced from 0.1 mm to 1.8 mm apart.

The cell body exchanges biological materials with the entire peripheral component of the neuron. The centrifugal system consists of production of acetylcholine, choline-acetyl transferase, and other substances in the cell body. These substances undergo proximodistal (centrifugal) transport toward the motor end plate (Ducker and Kauffman, 1977). There are at least 2 different rates of proximodistal axoplasmic flow, the more rapid of which is 17 mm per hour, or approximately 41 cm per day. In this flow, lipoproteins (Miani, 1963), proteins (McEwen and Grafstein, 1968; Ochs and Johnson, 1969; Sjostrand and Karlsson, 1969) neurosecretory material (Norstrom et al, 1971a and b) and catecholamines (Dahlstrom and Haggendhal, 1970) have been found to be fast moving components. The bulk of material, probably proteins, is transported at a much slower rate. This is probably of the order of 1 - 3 mm per day (Weiss and Hiscoe, 1948). This is the same velocity as the daily rate of elongation of axon sprouts in regenerating peripheral nerves (Guttman, 1958). While most of the peripheral axon and motor end plate nutrition comes from the cell body via this system, some of the distal portion of each fibre is dependent upon local blood vessels for nutrition. For this reason brain stem (nuclear) insults and/or local ischaemic lesions can impair peripheral nerve function or nerve regeneration.

Metabolic breakdown of neurotransmitters occurs at the motor end plate. Some of the breakdown products are thought to be carried back to the cell body via distoproximal (centripetal) axoplasmic flow. These flow rates appear to be slower than those associated with proximodistal transport.

When a parent neuron fires in the normal facial nucleus, the facial nerve axon undergoes depolarization, transmitting the impulse distally to the facial muscle fibres. Due to myelination of the nerve fibre, the wave of depolarization jumps from one node of Ranvier to the next, a process termed 'saltatory conduction'. This explains the characteristic rapid conduction velocity of the facial nerve (70 to 100 m/sec). After imperfect regeneration of the myelin sheath following injury, or in certain demyelinating lesions, conduction velocity is slowed markedly.

A second function of the myelin sheath appears to be that of providing electrical insulation between adjacent axons. Hence, when this myelin sheath is lost, short circuiting can occur between neighbouring fibres. Thus, the depolarization potential of one fibre may skip to a neighbouring fibre, producing secondary depolarization. The phenomenon is called ephapse and is thought to be one of the explanations for the spasms and synkinesis following imperfect nerve regeneration.

1.3 PATHOLOGY OF INJURY TO THE NERVE

The Cell Body and Proximal Axon

After section of the nerve, the first event is sealing off the nerve by degeneration to the next intact proximal node of Ranvier (Blumcke et al, 1966) An inflammatory reaction occurs. A few hours later, swelling of the axons in the proximal stump is seen.

The normal high level of metabolic activity in these neurons is increased markedly following axotomy. This is seen histologically as cellular swelling, nuclear eccentricity, nucleolar enlargement, and chromatolysis. These changes are associated with an important change in the intracellular enzymatic machinery. Neurotransmitter synthesis ceases and is replaced by the production of proteosynthetic materials (Weiss and Pillai, 1965; Ducker and Kauffman, 1977). Following axotomy, neurotransmitter substances decrease and proteosynthetic materials, such as glucose-6-phosphate dehydrogenase, ribonucleic acid (RNA), and nicotinamide adenine dinucleotide phosphate (NADPH) undergo marked increases. These enzymatic changes, and the histological change with which they are associated, are known as the 'retrograde reaction'.

There is axonal or neuroplasmic flow distally which is demonstrated when the nerve is sectioned (Weiss, 1967).

The term 'retrograde reaction' describes what happens proximally in the injured axon. However, the most important changes occur in the cell body and perhaps should be termed instead 'cell body response'. Cell bodies in the nucleus increase in size shortly following axotomy, although the cause for this change in size is not clear. There are significant changes in RNA synthesis which are thought to depend upon some process of gene activation. This increase in ribosomal RNA is thought to presage an increase in protein synthesis. Neurotransmitter production falls sharply following axotomy. This phenomenon cannot be explained by simple 'leakage' from the cut axon tip, since the overall result is a net increase in total protein synthesis. Other changes include proliferation of smooth endoplasmic reticulum, increased lipid synthesis, and certain changes in energy metabolism. Changes in axoplasmic flow are not observed until after the metabolic tranformation takes place in the cell body (Grafstein, 1975).

Other Nuclear Changes

During the time of maximal cellular swelling, there is a loosening of the attachment between the neuron and its neighbouring glia. Metabolic changes also occur in the dendrites. A significant retraction of the dendritic field has been observed. Dendritic loss of presynaptic boutons and depletion of transmitter associated protein have also been described (Kreutzberg, 1973).

The glial and interstitial fluid environment of the cell body is also altered markedly by peripheral nerve injury. Neighbouring microglial cells undergo mitoses and proliferation in the first week following nerve injury. These proliferating microglial cells cover most of the surface area of the cell body and its dendrites. This produces displacement of the terminal boutons. These boutons are removed by microglial processes, leaving the neurons with a reduced or absent afferent input. Changes in these supranuclear synapses may very well play an important role in the origin of mass movements, disturbances of fine movements, and effective movements. Loss or imperfect regeneration of inhibitory synapses might produce spasms of the facial nerve, while inappropriate regeneration of the synapses with 'mis-matching' would cause frank synkinesis.

In most cases the cell body recovers at about the same time as axon regrowth is complete to the periphery. The final condition of the cell body is influenced by the type of functional recon-nection made, eg whether the nerve is reconnected to a similar muscle fibre, or inappropriate regeneration occurs to a sensory end organ, glandular element, or other inappropriate distal structure. A significant observation, however, is that when reconnection of the regenerating axon is prevented, the decline in proteosynthetic materials occurs at the same time as it would have done if neural reconnection had occurred. This suggests that once the cell body reaction is initiated, it runs its course without feedback from the regenerating axon, presumably

according to a pre-set genetic programme (Grafstein, 1975).

The initiation of cell body response appears to be due to a 'signal compound' which is transported from the injury site proximally to the cell body. It is estimated that such a signal ascends the axon at a rate of several millimetres per day. The time required for the appearance of these changes is proportional to the distance between the cell body and the lesion (Ducker and Kauffman, 1977).

Factors Affecting Axonal Regeneration

Nerve repair results are superior in children possibly due to a superior ability for cerebral adaptation to a new afferent impulse pattern presented by misdirected axons or the shorter distances required to regenerate (Lundborg, 1987). Another possibility is the presence of growth hormone which is similar to nerve growth factor.

Tri-iodothyronine improves protein synthesis in the nerve cell body (Cook and Kiernan, 1976; Stelmack and Kiernan, 1977), the rate of axonal outgrowth (Cook and Kiernan, 1973) and maturation of regenerating axons (Stelmack and Kiernan, 1977).
L-thyroxinsodium (T4) increases the ability of axons to pass extended gaps (Danielsen et al, 1986).

Other factors which may have an effect on axonal growth but which

have not been conclusively proved are ACTH, pulsating electro-magnetic fields, inhibition of local scar formation by hormonal treatment, gangliosides which are normal membrane constituents located in the lipid layer of the plasma membrane, cyclic AMP, IGF (somatomedin-C), inhibitors of proetolytic enzymes, calcium inactivators to minimise degeneration in axon segments and irrigation fluids similar to axoplasm (Lundborg, 1987).

It is believed that the cell bodies of the neurones are dependent on a constant supply of neurotrophic factors (NTF) which can be synthesised by target organs of the nerve and/or by their corresponding Schwann cells. NTFs are transmitted by retrograde axonal transport along the axon and are used by the nerve cell body to sustain vital processes (Prestige, 1970; Landmesser and Pilar, 1978; Varon and Adler, 1980, 1981; Cowan, 1983). NTFs regulate the anabolic machinery of the axon but several other factors (neurite promoting factors - NPF), influence neurite outgrowth and elongation. These occur in the interstitium or are bound to the surfaces of the cells. Nerve growth factor is a NTF and is a protein with numerous analogues to insulin (Frazier et al, 1972; Varon and Adler, 1981).

As at the distal most part of each sprout, there is a growth cone which is a swelling from which several microspikes or filopodia arise. These explore the environment with constant movement. Considerable amounts of actin have been demonstrated in the filopodia, indicating an important role for their

mobility (Yamada et al, 1971; Wolosewick and Porter, 1976; Pleasure, 1980). Other factors influencing growth include the adherence of the substrate (Letourneau, 1979) and constant guidance by hard substances, eg cell surfaces or axons (Weiss, 1945).

It was originally believed that regenerating nerve fibres grow towards distal axons by a mechanism known as neurotropism or chemotaxis (Forsmann, 1898; Ramon and Cajal, 1928) but this was challenged by Weiss (1941) who felt this phenomenon was an effect of random axon growth. It would now appear that there is some degree of neurotropism shown by regenerating axons (Lundborg et al, 1986).

Axon regeneration does not occur immediately following peripheral nerve injury (axotomy). It takes approximately 3 weeks for these changes to reach maximal levels. Some investigators feel that this period represents the optimal time to wait before performing reparative nerve surgery (McCabe, 1977).

Animal research has shown that trimming of the severed nerve ends at 3 weeks produces a secondary increase in axoplasmic transport rates (McQuarrie and Grafstein, 1973). Other investigators, however, notably Sunderland, feel that such a trimming of the proximal stump at the injury site necessitates a second 'retrograde reaction' and results in a secondary decrease in axon regeneration rates (Sunderland 1980).

1.4 CLASSIFICATION OF INJURY

In the early 1940s, Seddon produced a classification of nerve injuries that was quite useful at the time: neurapraxia, axonotmesis, and neurotmesis. The classic description of these injuries, however, was somewhat difficult to correlate with the clinical situation (Seddon, 1943).

Neuropraxia

This is temporary cessation of conduction of the nerve with no loss of axon continuity. The effect of this is entirely transitory once the aetiological factor has been removed and recovery is complete, eg the Friday night drunk falls asleep with his arm over the back of the chair and on awaking finds his arm and hand temporarily paralysed.

Axonotmesis

The axons themselves are damaged leading to distal axon destruction known as Wallerian degeneration. This means the neural elements die and the myelin sheath is digested by Schwann cells in about 7 days. Macrophages remove all the debris. Eventually axonal regeneration takes place at about 1 mm to 4 mm per day. Scar tissue is not a feature and recovery is satisfactory. Examples of this are mild compression, cooling below $5^{o}C$ for 3 minutes or transient ischaemia.

Neurotmesis

The nerve fibre is severed or the endoneurium is severely disrupted amounting to total physiological severance. The axon and surrounding connective tissue are damaged but occasionally the fascicular architecture is retained. Regeneration is much less complete than the lesser forms of injury.

In axonotmesis and neurotmesis the axon degenerates, Wallerian degeneration occurs and eventually regeneration may occur. Frew (1979) added 2 more possibilities to the above classification:

Maximum Nerve Damage

This indicates more extensive injury involving the axon connective tissue and the perineurium. This resultant regeneration is poorly orientated and much less effective than with any of the previously described degrees of nerve damage. The nerve may well be microscopically intact after this insult.

Nerve Division

The nerve may not only be severed but there may be a gap if a portion of the nerve has been removed by injury. A graft may be required to close this gap.

Sunderland suggested a more anatomical classification in 5 degrees of the damage to an individual axon.

First Degree Injury (Conduction Block)

This type of nerve fibre injury is analogous to Seddon's 'neurapraxia' and is also called conduction block injury. The axon and its endoneurial tube are slightly indented, twisted or otherwise distorted. The Schwann sheath, myelin layer, endoneurium and axon are all in continuity. Both distoproximal and proximodistal axoplasmic transport continue. The nerve fibre distal to the site of injury retains normal electrical response. Normally propagated waves of depolarization from the cell body, however, and electrical stimulation proximal to the site of lesion, evoke no distal response, since conduction is blocked.

If all 7000 motor fibres in one facial nerve are in a first degree of injury, the nerve can be electrically stimulated. In fact, the distal facial nerve should stimulate at the same level of electrical stimulation as the normal opposite side. Since all 7000 endoneurial tubes (Schwann sheath and myelin layer) are intact, the facial function will be fully restored when the block is cleared. There will be no permanent sequelae from such an injury.

Recovery in first degree injuries is usually quite rapid, most commonly less than 3 weeks, since the cell body does not have to regenerate a peripheral axon.

Second Degree Injury (Loss of Axon Continuity with Preservation of Endoneurial Tube)

A second degree injury results from an increasing force of indentation, twisting or distortion. In this injury the axon is compressed to the extent that continuity is disrupted.

Neurotransmitter substances manufactured in the cell body can no longer reach the motor end plate region, and neural metabolites from the muscle and motor end plate cannot pass proximally to the cell body. This loss of axon continuity makes a second degree injury much more detrimental to the entire neuron than a first degree injury for the following reasons:

(a) A 'biological signal' tells the cell body that the distal axon has been interrupted.

(b) This invokes the 'metabolic transformaton' described earlier.

(c) The distal axon undergoes Wallerian degeneration.

(d) Various biochemical and histological alterations occur in muscle and in the brain stem due to this denervation.

The proximal axon segment now acts as a communication conduit from cell body to site of injury. The proximal segment retains

its endoneurial tube structures, as does the distal segment in this degree of injury. This proximal segment will become (in 14 to 21 days) a conduit for proteosynthetic substances which will allow axon regeneration (but the distal axon must regenerate to the motor end plate for recovery to occur).

At the site of lesion the endoneurial tube retains continuity, although there is often some thinning of the myelin layer. The perineurium surrounding the fascicle, and the fascicular arrangement of the nerve, remain intact. Retention of endoneurial tube continuity allows the nerve to regenerate to its former identical muscle fibre(s), and permits normal neuromuscular function, provided that axon regeneration is complete. Clinically, recovery begins at 3 - 8 weeks and eventually recovery is slightly incomplete.

Axon Regeneration

There is concomitant axonal degeneration and regeneration in the form of axon sprouting (Van Boek et al, 1982)

Ordinarily, the axon sprouts do not enter the distal segment for a period of 7 to 21 days. The mechanisms for this regeneration are incompletely understood. It appears that with distal lesions this sprouting may occur somewhat later, although regeneration will be more complete since the neuron has sustained less of an amputation injury. In proximal lesions axon sprouting may occur

earlier, provided that the more proximal injury does not result in death of the cell body (Kreutzberg, 1982).

Once axon sprouting and growth begin, the new axons grow at the rate of approximately 1 mm per day. The distance from injury to the motor end plate, nutritional factors, age and type of injury all affect the length of the recovery period and the quality of resultant function. Blunt injuries appear to induce a heightened cell body response, and more likely to result in neuron death than sharp transections.

Location of the injury appears to be an important factor in determining extent of central changes. Proximal injuries (cerebellopontine angle, meatal portion, labyrinthine portion) cause greater nuclear change because a larger portion of the neuron is amputated. In contrast, a laceration of a small distal facial nerve branch leaves the long axonal process intact, requiring less regeneration for the nerve to return to its motor end plate (Ducker and Kauffman, 1977).

All major classes of peripheral axons regenerate and form new synapsis after axotomy. Axons may also regenerate through some cranial nerves to form new synapsis or neurones in the brain. In mammals, severed axons make synapsis near the site of injury. When part of the nerve supply to a peripheral target is removed the remaining axons sprout with processes and form new synapsis on nearby denervated cells. Axons reinnervating denervated

skeletal muscle show a preference for original synaptic sites Nichols, 1982).

Reinnervation guidance is poorly understood but possibly remaining perineural tubes serve as a guide. Axons outside these also prefer to reinnervate the original sites. Reinnervation remains precise when the active sites of acetylcholine are blocked by antagonists or after prolonged deinnervation. Myofibre itself is not required for precise reinnervation of original synaptic sites; possibly there is some attraction of the synaptic basal laminae of the basement membrane sheaths (Purves, 1982).

An ectopic synapsis forms when a nerve is denied access to original end plates, either because it is implanted too far from the end zone or because access to original end plates is blocked by, eg connective tissue. Muscle activity prevents hyper innervation of the muscle by 'foreign' nerves but if the 'original' nerve is damaged then the foreign nerve grows into the muscle.

When muscle fibres are denervated, new acetylcholine receptors are synthesised and inserted throughout the muscle fibre membrane. These are super sensitive to acetylcholine. Thereafter protein synthesis diminishes and protein degradation increases, leading to muscle atrophy.

Denervated muscle releases substances to induce axon sprouting and degenerating nerve releases neurocletin to induce axon sprouting.

Not all synapsis formed after nerve injury are maintained and there is an element of competition between sprouting axons and 'foreign' nerves. Therefore, 4 classes of factors which regulate regeneration of nerve and muscle:

(a) muscle activity

(b) soluble agents

(c) cell-surface or extracellular matrix components

(d) products of nerve degeneration

The substance which emanates from the nerve trunk which attracts regenerating axons has been termed nerve trunk growth factor. Motor nerve growth factor is produced by muscle, particularly following denervation. This would tend to attract the axons to the muscle to enhance reinnervation. Also neural cell adhesion molecule is a membrane glycoprotein present on the surface of most peripheral and central neurones which also attracts reinnervating axons (Janecka, 1987).

Marked species differences are known to exist with regard to nerve regeneration. Certain lizards will regrow entire segments of spinal cord when their tail has been amputated. Rats and dogs have less regenerative potential, while baboons, chimpanzees, and man are least capable of nerve regeneration.

Certain types of nerve wounds (massive blast injury, war injuries) may result in devascularization of the proximal and/or distal peripheral nerve. This kind of wound promotes excessive scar tissue and delays the healing, both of which adversely affect subsequent nerve regeneration. Patients with multiple injuries may become catabolic for 2 to 3 months and this, too, adversely influences nerve regeneration.

Even after the regenerating axon reaches the periphery, the fibre is not ready to conduct normally until the cell body switches its enzymatic machinery back to those biochemical processes concerned with excitation and conduction. This takes from 60 to 90 days following the completion of axon regeneration.

It is probable that metabolic factors in the denervated muscle promote axon sprouting. This results in a change in configuration of motor units (a motor unit is defined as the muscle fibres innervated by one motor neuron) and corresponding changes in electromyographic patterns.

Third Degree Injury (Disruption of Axon and Endoneurial Tube)

Third degree injury results from a greater force of indentation, twisting, or distortion than first or second degree injuries. In this injury the axon and the endoneurial tube become discontinuous.

There is Wallerian degeneration similar to a second degree injury. During axon regeneration the tiny axon sprouts are free to enter any distal endoneurial tube available, curl up in between distal endoneurial tubules, or wander elsewhere within the nerve fascicle. This allows one or more of the following: (1) one axon sprouts to enter the correct endoneurial tube; (2) another axon sprout (from the same proximal axon) to enter an 'incorrect' distal tubule; (3) loss of many axon sprouts which fail to enter any tubule.

If axon regeneration is complete, distal muscle strength may be nearly normal, except that 'mass movements' or synkinesis, will develop because proximal axons no longer pass distally to the same muscles as prior to injury. These injuries begin to regenerate at 2 - 4 months but only progress to moderate or poor recovery.

Fourth Degree Injury

Fourth degree injury is defined as disruption of perineurium surrounding nerve fascicles, with preservation of the nerve sheath. In this type of injury, a force is exerted on the nerve sufficient to rupture the fascicular containment layer, the perineurium. All axons within the fascicle undergo third degree injury, while the fourth degree is added to indicate that a greater force was needed to cause the injury, and that a poorer results will follow nerve regeneration.

As perineurium no longer confines regenerating axon sprouts to their appropriate fascicle, they are free to regenerate into the interfascicular sheath and to be lost in neuroma formation, or to regenerate into a distal endoneurial tubule within an adjacent fascicle. This results in a decreased axon population at the periphery and therefore a 'less concentrated' innervation of the facial muscle. Possibly the fewer axons reaching the periphery sprout are sufficient to innervate all the motor end plates previously innervated, but an insufficient number of axons will usually result in less muscle contraction following this degree of injury. This type of injury takes 4 - 18 months to show recovery with eventual quite incomplete recovery.

Fifth Degree Injury

If the nerve trunk is entirely disrupted the lesion is termed fifth degree or neurotmesis. This means that, in addition to disruption of the axon, endoneurium and perineurium, the sheath surrounding the nerve has been torn. Again, all axons of the nerve will undergo Wallerian degeneration and subsequently mount a regenerative effort. However, in this injury axon sprouts are not only free to regenerate to the periphery, as with the other degrees, but also have the potential to regenerate outside the nerve and be lost in extraneural gliomata. This is the type of injury seen with all sharp complete transections. Naturally, these injuries will result in synkinesis and muscle weakness and usually show no evidence of recovery at one year.

1.5 DEGREE OF INJURY

If nerve injuries are always purely first or second degree, the results of nerve excitability testing would be easy to interpret. If all 7000 fibres had undergone a first degree injury, electrical excitability on the face would be normal. If all 7000 fibres had sustained a second degree injury, electrical excitability would disappear between 48 and 96 hours. This would also be true for third, fourth, and fifth degree injuries.

Most injuries to the facial nerve contain a mixture of the various degrees. The effects of these mixed lesions on excitability testing are not yet known. However, since excitability testing is normal with all fibres in the first degree of injury and absent if all fibres are in a second degree of injury, most patients who have intermediate results with nerve excitability testing probably have mixed lesions. This is the type of lesion which may allow so-called 'immediate return of function' following facial nerve decompression (McCabe, 1977). This could hypothetically result from releasing the conduction block of the fibres in first degree of injury which allows them to resume normal function.

It is, however, a somewhat facile concept to expect that all 10,000 axons of the facial nerve suffer exactly the same type of lesion (except in the complete transection of a Type 5 injury). Most injuries thus contain a mixture of injury types which may

produce confusing and conflicting results from electrical testing and clinical recovery (Crumley, 1984c).

The Motor End Plate Region

Facial nerve axons normally innervate a number of muscle fibres which is intermediate between the huge motor units of the extremity muscles (2000 muscle fibres per axon in sartorius) and those of the extraocular muscles or laryngeal muscles (2 to 10 muscle fibres per axon). When a peripheral axon undergoes transection, all its motor end plates change their biochemical composition. These changes are somewhat similar to those described in the nerve cell body in the brain stem. An increase in muscle ribonucleic acid, ribosomes and rough endoplasmic reticulum is seen in the early denervation period. This allows proteosynthesis, which may become necessary because of the breakdown of contractile proteins in the muscle cell. It has also been shown that the new ribosomes may be synthesizing a protein receptor substance for acetylcholine, since it is well known that acetylcholine sensitivity spreads to cover the entire muscle fibre following denervation, rather than being concentrated solely at the motor end plate zone and acetyl choline receptor concentration increases in this situation (Sanes et al, 1980; Arglebe et al, 1981).

Certain substances elaborated from the motor end plate region are known to induce axon sprouting following denervation.

Neurocletin and a substance similar to nerve growth factor have been described as promoting axon sprouting in this region (Hoffman, 1950). It is known that these substances will promote axon sprouting from a normal distal nerve fibre towards a denervated muscle fibre (Diamond et al, 1976). An 'axon sprouting inhibitor factor' has also been described. This biochemical substance, normally found near the motor end plate, prevents axon sprouting in the intact neuromuscular unit. Following axotomy, the absence of this factor is thought to allow axon sprouting, as does the active elaboration of the 2 substances described above.

If no neural regeneration occurs, the muscle fibre may (1) be reinnervated by adjacent muscles and/or nerves via axon sprouting; (2) remain quiescent in a denervated state for a period of time, expectantly awaiting the arrival of reinnervating axons; (3) undergo atrophy (following a period of time), and eventually even disappear and be replaced by fibrous tissue.

The length of time from denervation to atrophy is highly controversial. Basic investigations have concluded that the time before severe atrophy occurs may be as short as 2 to 3 years. However, clinical observers have performed successful reinnervation surgery as late as 15 to 20 years following paralysis, indicating that the denervated muscle was preserved. The reason for this discrepancy is most probably that neural regeneration following severe nerve injuries is inadequate to induce actual

muscle contraction, yet new axon sprouting is capable of occupying the motor end plates and transporting the necessary supply of neurometabolites to the motor end plate region from the cell bodies in the facial nucleus. In that instance, the muscle fibres would be 'preserved' until a reinnervation procedure or resolution of the proximal nerve injury allowed axons to regenerate into the muscle. An extremely important aspect of this phenomenon is that innervated muscle fibres, even though innervated by axons too 'diluted' to produce movement, will not accept new innervation. Hence it is probably important to transect this pre-existing innervation prior to introducing a neural reinnervation source.

Central Phenomena

The effects of peripheral injury on the facial nucleus and supranuclear afferent systems are only partially understood. Kreutzberg (1973) has described increased mitotic activity of perineuronal cells following facial nerve axotomy. Microglial processes from such cells appear to cover the surfaces and synaptic terminals of such facial nerve cell bodies. It is not clear what the end result of these nuclear changes might be, but it is possible that synaptic 'mismatching' occurs following restoration of the cell body to neural transmission (Kreutzberg, 1973) or disorganisation of the structure of the cell nucleus (Thomander and Aldoskoglus, 1984).

It is well known that the facial nucleus has multiple supranuclear afferent systems acting upon it. Voluntary facial movements ('show your teeth'; 'wrinkle your forehead') originate in the precentral gyrus of the cerebral cortex. These corticobulbar fibres pass downward through the posterior part of the internal capsule and most then cross the caudal pons to synapse in the opposite facial nucleus.

Emotional (involuntary) movements, such as smiling, laughing or eye blinking, are mediated by the hypothalamus, globus pallidus, interneurons in the reticular formation, and brain stem reflex arcs. This explains how the patient with a cerebral vascular accident and infarction of the internal capsule may smile spontaneously when amused, yet be unable to voluntarily 'show his teeth'. Conversely, patients with postencephalitic Parkinsonism (lesions of the globus pallidus) frequently can voluntarily move their faces normally but show no emotional involuntary movements. Other supranuclear afferents affecting the facial nucleus include those from the superior colliculus of the optic system (blink reflex), the superior olive of the auditory system (stapedius reflex), sensory trigeminal nucleus (corneal reflex), and the nucleus of tractus solitarius (chewing and sucking following introduction of food into the mouth) (Crumley, 1979).

Since it is presumed that each facial nerve cell body derives input from several if not all of these sources, mismatching of

these supranuclear inputs might well result in spasms and synkinesis seen following peripheral nerve injuries. This provides yet another possible explanation for synkinetic movements following peripheral nerve injuries.

1.6 PATHOPHYSIOLOGY OF NERVE INJURY

Ge et al (1982) crushed the buccolabial branches of the guinea pig facial nerves to produce axonotmesis, Wallerian degeneration, and demyelination. The animals were allowed to live from 1 - 8 weeks after surgery and the lesions examined by transmission electron microscopy, electrophysiologic tests, and cytochemical staining methods to identify sodium channels.

At 2 weeks the buccal nerve demonstrated a variable conduction block to electrical stimulation. Between the second and sixth weeks, the axolemma was reconstituted without remyelination: The regenerated, unmyelinated axon had a smaller diameter which presented less surface area to an electrical stimulus, thereby, preventing electrical loading and facilitating conduction. Diffuse sodium channels aggregated into high-density clusters from the proximal end of the distal axonal stump along the length of the demyelinated axolemma, a precursor to saltatory conduction. These researchers noted that remyelination was complete in many distal axon segments by 8 weeks, when electrical stimulation returned to normal thresholds for muscle contraction and that reconstituted nodes of Ranvier had shorter internodal distances in the regenerated axon which might have facilitated saltatory conduction during initial remyelination.

Schuknecht and Shinozake-Muri (1985) studied 12 temporal bone specimens that represented a spectrum of facial nerve disorders

and sites of lesion, including intracranial neoplasms (astrocytoma, meningioma, schwannoma), intratemporal lesions (schwannoma, petrositis, osteopetrosis, herpes zoster oticus) and extracranial salivary gland neoplasia among others. The study correlated histologic features of the lesions with degenerative changes in the facial nerve. The motor and sensory divisions of the facial nerve adapt well to space-occupying lesions in the internal auditory canal but are highly vulnerable to pressure in the Fallopian canal. In fact, destruction of the motor division at any location in the canal caused atrophy distal to the lesion. Destruction of the sensory division medial to the geniculate ganglion spared sensory fibres distal to the geniculate ganglion, while a destructive lesion distal to the geniculate ganglion caused atrophy of sensory fibres distal to the lesion.

It was formerly thought that in the circumstances of subacute compression, eg when compression of the nerve continues for several minutes or hours, that nerve damage is ischaemic and secondary to occlusion and rupture of the intraneural blood vessels following their compression (Denny-Brown and Brenner, 1944).

However, recent experimental studies of tourniquet paralysis in the baboon have demonstrated direct mechanical distortion of nerve fibres (Ochoa et al, 1972).

Ranvier's nodes and their attached myelin sheaths are displaced away from the compression site and towards uncompressed tissue, leading to the invagination of one internode into the next and finally to paranodal demyelination. If recovery is allowed by release of compression, the demyelinated lengths of axon are remyelinated in short intercollated segments. The large myelinated nerve fibres are preferentially involved with sparing of the small myelinated and non-myelinated fibres. This characteristic lesion has been observed in sub acute compression of the median nerve in man (Neary et al, 1975). Occasionally pressure palsy may be the presenting feature of an underlying subclinical and generalised polyneuropathy, ie diabetes, alcoholism and the Guillan-Barre syndrome. The susceptibility to recurrent pressure palsies may be inherited as an autosomal dominant in some families (Earle et al, 1964) and sural nerve biopsy in such patients reveals the presence of demyelination and remyelination and localised sausage-shaped swellings of the myelin sheaths (Behse et al, 1972).

Entrapment Neuropathy

Pathogenesis

The guinea pig has provided an experimental model for the study of entrapment neuropathy because a caged animal develops compression of the median and ulnar nerves under the transverse cartilaginous bar which supports the footpad. Ochoa and Marotte

(1973) described a characteristic sequence of pathological changes in the compressed nerve fibres which was thought to be due to the effect of repeated pressure waves set up at the site of entrapment and spreading up and down the nerve fibres away from this site and towards uncompressed tissue. This mild but repeated compression of the larger myelinated nerve fibres results in detachment of the myelin lamellae from their normal site of attachment at Ranvier's node so that loops of myelin slip down the axon away from the site of entrapment. This myelin slippage leads to thinning of the myelin sheath at one end of the internode. At the other end the myelin lamellae pile up in redundant folds giving an expanded or bulbous appearance. The orientation of these polarized changes of the internodes is reversed on either side of the entrapment site corresponding to the direction of the pressure waves. With further myelin slippage the paranodal axon is demyelinated. However, in the early stages of the lesion remyelination is possible and short thinly myelinated intercalated segments replace the areas of bare axon. At increasing distances from the entrapment site the nerve fibres and their internodes return to a normal appearance.

Studies in man have shown at autopsy the occurrence of this characteristic lesion in the median nerve at the wrist and ulnar nerve at the elbow in patients who had been free of neurological symptoms during their lives (Neary et al, 1975). The finding of this subclinical entrapment neuropathy suggests that even in clinically normal subjects, a long-standing process of

demyelination and remyelination may be taking place in the larger myelinated fibres at entrapment sites.

In more severe examples of compression neuropathy in the guinea pig, extensive demyelination of large myelinated nerve fibres occurs at the entrapment site (Marotte, 1974) and demyelination was found to be the primary pathological process in a study of entrapment neuropathy of the ulnar nerve in man (Neary et al, 1975).

Degeneration of nerve fibres as a result of compression is not a striking early pathological feature in animals or man and the presence of regeneration clusters of small thinly myelinated axons at the entrapment site suggests a continual process of degeneration and regeneration with the former predominating in more severe cases of nerve compression.

Thus with increasing duration and severity of nerve compression demyelination and later degeneration of nerve fibres appear to outstrip compensatory remyelination and regeneration. Presumably the interplay of these pathological processes determines whether the entrapment neuropathy remains subclinical or becomes apparent and accounts for the potential for recovery following decompression of the nerve.

As well as the abnormalities in the nerve fibres themselves, pathological changes in the interstitial tissues of the trapped

nerve may contribute to the symptoms and signs of entrapment neuropathy.

At and just proximal to the entrapment site, the nerve is swollen and this neuroma is the result of a thickened epineurium and perineurium together with endoneurial swelling. The normal fascicular architecture of the nerve is lost because of the disruption of the perineurium so that the nerve fibres lie dispersed, separated by excessive proteinaceous endoneurial fluid. The perineurium usually acts as a diffusion barrier to protein molecules and when damaged it may become more permeable and allow proteinaceous fluid to enter the endoneurium (Olsson et al, 1971). The distal flow of this endoneurial fluid down the nerve may be obstructed at the entrapment site giving rise to an excessive accumulation of oedema proximally. Excessive endoneurial oedema collecting within a thickened and unyielding casement of epineurium may lead to a rise in intraneural pressure and thus add to the compressive forces of extraneural origin. Furthermore, if the biochemical constituents of this fluid are abnormal the effects of altering the extracellular milieu of the nerve fibres may lead to a biochemical rather than a mechanical embarrassment of nerve function.

The amount of endoneurial oedema may be expected to vary thoughout the day in response to the effects of stasis of the limb, gravity, and changes in limb blood flow. Such episodic fluctuations may explain the episodic sensory symptoms of

entrapment neuropathy which may also be provoked by tourniquet compression of the limb. Alterations in the amount of endoneurial fluid in the nerve may account for the response to treatment with local and systemic corticosteroids and the often rapid disappearance of symptoms such as pain and paraesthesiae following decompression of the trapped nerve.

Clinical Features

The specific symptoms and signs of entrapment neuropathy depend upon the particualr peripheral nerve or nerve root involved.

The onset of symptoms is usually gradual but may be rapid. Symptoms, especially those of pain and paraesthesiae, may be paroxysmal and indeed the syndrome itself may be transient, for example if occurring during pregnancy or when due to hypothyroidism (the carpal tunnel syndrome). Symptoms of motor dysfunction are more often chronic and progressive.

Motor Dysfunction

Weakness of a group of muscles may be the presenting symptom of entrapment neuropathy and the patient may be observant enough to notice wasting of specific muscles. Occasionally, the muscles supplied by a nerve trunk are not all equally affected by compression. For example, in the thoracic outlet compression syndrome, the small muscles of the hand supplied by the median

nerve are often more severely involved than those supplied by the ulnar nerve and the striking wasting of the abductor pollicis brevis muscle may lead to diagnostic confusion with the carpal tunnel syndrome.

Recognition of the differential involvement of the small muscles of the hand is particularly important in distinguishing between the thoracic outlet compression syndrome, the carpal tunnel syndrome, and ulnar neuropathy (at the elbow and in the palm). Nerve root compression (the thoracic outlet compression syndrome) leads to weakness of all the small muscles of the hand, whereas the exclusive affection of the abductor pollicis brevis muscle (the carpal tunnel syndrome) or the interossei muscles (ulnar neuropathy) points to entrapment of the appropriate peripheral nerve.

Sensory Dysfunction

Sensory symptoms may be positive (pain and paraesthesiae) or negative (numbness), the former often denoting the presenting and occasionally the only features of the disorder. The site of the pain and paraesthesiae may not indicate the point of nerve entrapment because symptoms are felt outside the cutaneous sensory distribution of the nerve; for example, the pain arising from the carpal tunnel syndrome can radiate into the forearm, the upper arm and even the shoulder. More helpful in diagnosis is a consideration of the factors that precipitate these

symptoms. Nocturnal acroparaesthesiae are especially common in the carpal tunnel syndrome in which pain and paraesthesia may be brought on by wringing movements of the hands, whereas symptoms of the thoracic outlet compression syndrome are provoked by traction on the arm during carrying or by prolonged elevation of the arms during housework (Lascelles et al, 1977; Le Quesne, 1978).

In contrast to these positive symptoms the site and extent of numbness reported by the patient is of greater value in anatomical localisation of the site of nerve entrapment and corresponds to the cutaneous sensory supply of the nerve. When examining for sensory loss, light-touch sensation is often found to be more severely and extensively involved than that to pin prick and when the hand is the site of involvement, the differential affection of 2 point discrimination in the fingers can be helpful diagnostically. The 'splitting' of the ring finger by preferential sensory loss is seen in peripheral nerve lesions (for example, in the median or the ulnar nerve) but not in root disorders (the thoracic outlet compression syndrome).

Associated Abnormalities

Nerve Disorder

Sometimes thickening of a nerve may be felt immediately proximal to its site of entrapment (for example the ulnar nerve at the

elbow) but it is a sign often mistakenly attributed to a normal nerve by the unpractised. Palpation or percussion of the trapped nerve may produce characteristic pain and paraesthesiae. Sometimes manoeuvres that lead to increased compression of the nerves with resulting pain and paraesthesiae may be of diagnostic help. For example, traction on the arm and forcible depression of the shoulder may reproduce the symptoms of the thoracic outlet compression syndrome. The prolonged application of a tourniquet to the limb may bring on the symptoms of carpal tunnel syndrome.

1:7 NEUROPHYSIOLOGY OF NERVE INJURY AND NERVE EXCITABILITY TESTS

Nerve conduction tests on the facial nerve remain relatively contentious. There are a variety of different methods of testing the nerve, none of which have achieved total acceptance. Indeed, for the prognostication of the recovery of a Bell's palsy, many authors have concluded that electrical stimulation tests were of little value (Campbell et al, 1962; Groves and Gibson, 1974; Olsen, 1975).

Strength Duration Curve

The minimal intensity of an effective stimulus of any length of duration of greater than 100 ms is called the rheobase. The chronaxie is the time required for a current twice the rheobase to produce a response.

Determining the strength duration curve necessitates tedious and somewhat painful methodology and thus has not gained wide acceptance as a clinical test. Briefly, this curve depicts the relation between amplitude and duration of a stimulating impulse at the threshold for muscle response. If denervation has occurred, a stronger than normal stimulus is needed to activate the muscle, and the shape of the strength duration curve is thus changed.

Minimal Nerve Excitability Test

The minimal nerve excitability test requires simple percutaneous stimulation of the facial nerve at the stylomastoid foramen while raising the intensity of a short duration current to the threshold at which a just visible muscle contraction is observed (Jongkees, 1977). Both the affected and unaffected side are then stimulated and the 2 compared. Because neural degeneration is often neither acute nor complete, a single examination is not sufficient: re-examination days or weeks later is necessary to obtain accurate results.

Maximum Nerve Excitability

Since the reliability of minimal nerve excitability has been challenged, a modification called the maximum nerve excitability test has gained popularity (May, 1977). In both tests, the indifferent, ground electrode is moistened with conducting paste, placed on the back of the patient's hand, and held by the patient. This facilitates testing and 'gives the patient something to do'. The observer is placed so as to see both sides of the face simultaneously. The testing, stimulating probe is applied to the nerve branch to be tested at the current intensity that produces a just visible muscle twitch. When the first contraction is observed, the area is explored to find the most sensitive point, displaying the maximal amount of muscle motion. The current is then increased 1 or 2 mamps above this threshold

to obtain to obtain maximal nerve excitability stimulation. Test results are expressed as the difference in facial muscle movement when comparing the affected side of the face with the normal side; those results are recorded as equal or decreased movement. Additionally, when muscle response to maximal nerve excitability stimulation is decreased, the observer notes whether this decrease indicates minimal, moderate, severe, or complete denervation. May (1977) feels that the strength of the muscle response is dependent on the number of functioning axons.

Although facial nerve excitability testing is a simple procedure, determining the location of the peripheral branches requires experience. The branch to the frontal muscle is usually found approximately 2.5 cm posterior to the outer canthus of the eye. The branch to the orbicular muscle of the eye is stimulated at the lateral border of the orbit. Location of the branch to the orbicular muscle of the mouth varies the most of all three branches, but is usually just anterior to the notch where the facial artery traverses the mandible. The stimulating probe may need to be moved in order to determine the point of maximal response, as the facial nerve can branch in many directions beyond the stylo mastoid foramen.

Electroneurography

Electroneurography (ENOG) is the objective electrophysiologic measurement and recording of a muscle compound action potential

used to assess the integrity of the facial nerve. The test has been called electroneuronography, neuromyography, and evoked electromyography. However, the original term electroneurography (Esslen, 1977) and abbreviation ENOG are sufficiently entrenched in the literature to be acceptable.

The stimulating electrode is placed over the nerve trunk at the stylomastoid foramen and the surface recording electrode over the muscle group to be tested, ie orbicularis oris or orbicularis oculi. The nerve is stimulated with a supramaximal stimulus, ie the voltage required to produce a maximum facial movement is noted and a stimulus of 20% more is used to ensure that all nerve fibres are stimulated. Functional status of the nerve is assessed by comparing amplitudes of the compound muscle action potentials. The size of the muscle action potential can be accurately measured and is an acceptable indication of nerve function. This method is considered by some an improvement of the maximal nerve excitability test. Instead of estimating by visual inspection the effect of nerve stimulation, the muscle action potential is recorded. Obviously, for an intratemporal lesion, adequate time must be allowed for Wallerian degeneration to pass distal to the stimulating electrode, ie 2 to 3 days before performing the test.

Kartush and associates (1984) examined, qualitatively, the variations in instrumentation and technique that may account for interside and intertest variance, and reviewed sources of inter-

pretive error. They compared fixed, standardised electrode positioning results (Hughes et al 1983) with 'optimised' positioning results, in which the stimulating and recording electrodes were held by hand and were manipulated during each test trial to positions that elicited maximal responses.

No difference was found in intertest variance with optimal (17.8%) versus standardised (19.5%) placement, but interside variance (right versus left) in normal subjects was less with optimal (10.4%) than with standarised (21.2%) placement. The contention that standardised, fixed electrode placement minimises potential error is outweighed by the more reliable responses obtained with 'optimised' electrode placement, particularly in recording reponses of degenerated nerves. Smith et al (1988) found that the placement of the stimulating electrode was critical and that the latency could be used to check the equal stimulation of both facial nerves.

Esslen (1977) recommends waiting until the twentieth stimulation before recording the response, to allow skin resistance and the 'motor unit volley' to achieve optimal condition. However, in a recent study of 24 normal subjects by Gavilan and colleagues (1985) there was no significant difference in responses 1 to 5 versus 20 to 25, thus concluded that test - retest variability (3.1%; range 0.9 - 10.5%) did not result from desynchronisation of the motor unit volley. In addition, such minimal variability did not warrant computer averaging.

This 'desynchronisation of the motor unit volley' remains a nebulous yet undoubtedly real phenomenon. It is hoped that future research will unravel its mysteries; in the meantime, the phenomenon helps explain absent electrical responses in patients with less than complete paralysis.

A newer potential application of ENOG deals with preoperative assessment of tumours of the temporal bone. Kartush and associates (1986) studied 82 patients with temporal bone and cerebellopontine angle tumours. Of 65 patients who had normal preoperative facial function, 32 (49%) demonstrated significant amplitude reduction on preoperative ENOG testing: a 20% reduction in amplitude reliably predicted facial nerve involvement by tumour, except when infection was present. However, ENOG was not an accurate predictor of postoperative facial function. Kartush et al (1986) suggests that preoperative ENOG information might aid in patient counselling and provide medicolegal documentation if necessary when nerve involvement by tumour is found to be subclinical.

Intraoperative monitoring of facial nerve function was described by Metson et al (1985). Active electrodes are placed at the vertex and ipsilateral mastoid. The contralateral mastoid has a ground electrode. A monophasic negative evoked potential is generated from the facial nerve at the stylomastoid foramen and is repeated as required when the facial nerve is under threat at surgery.

About 14 to 21 days after denervation, the muscle exhibits fibrillation potentials. These are measured by electromyography introduced by Weddel in 1940 (Cull, 1989) but, because they are a late phenomenon, they are of little use in treatment.

1.8 TRAUMA

Temporal Bone Fractures

The temporal bone is a thick strong bone which resists external trauma. To disrupt it requires a deforming unilateral force of considerable magnitude or a bilateral force which squeezes the skull on both sides, producing a shearing effect on the temporal bone. Gapany (1985) experimentally produced fractures in cadavers and found a direct force to the temporal bone produces a longitudinal fracture. Occipital mastoid trauma produces a transverse fracture.

Frew (1979) reports an incidence of 4% of head injuries in Newcastle. Bebear (1984) however, from 718 cases of fracture of the temporal bone, found 374 had a facial palsy. The series by Hough (1973) showed that ossicular injuries and displacements after a skull injury could only occur with considerable bony distortion. Cawthorne (1956) found 6 cases out of 7 with a longitudinal fracture had also dislocated an incus. Fractures of the petrous temporal bone are said to occur in 75% of all base of skull fractures. Many base of skull fractures are, however, not radiologically evident and are, therefore, not included in this figure.

Types of Fracture

Traditionally fractures of the temporal bone are classified as longitudinal or transverse. The longitudinal fracture (90% of temporal bone fractures) line runs obliquely from above through the squamous part of the temporal bone, longitudinally along the posterior surface of the pyramid often involving the middle ear and leaking cerebro spinal fluid. The labyrinth may be bypassed (Leonard and Belafskey, 1973). In the transverse fracture (10%) the line runs vertically through the squamous petrous bones, sometimes disrupting the lambdoid suture, into the labyrinth. It extends across the labyrinth and involves the jugular foramen, vestibule and internal auditory meatus. It is often Y-shaped producing a triangular wedge of fractured bone. Fisch (1979) describes how in 70% of transverse fractures, the limbs of the fracture produce a double lesion of the labyrinthine segment of the Fallopian canal in the tympanic segment and, therefore, requires a good exposure of all of these in reduction. Grove (1939) distinguished 2 types of transverse fractures. The inner transverse fracture which involves the internal auditory meatus and cochlea and the outer transverse which destroys the soft parts of the labyrinth. Many fractures are combined longitudinal and transverse. Ramadier and Causse (1937) have described the fracture of the mastoid tip not involving the Fallopian canal. Fisch (1980) found that temporal bone fractures are the second commonest facial nerve palsy that he sees.

In Guerrier's (1967) series of 2000 head injuries, 15% had acute ear problems from the trauma. Briggs and Potter (1971) found that if the temporal bone fracture extended into the middle ear, a delayed seventh nerve paralysis may occur in up to 39%.

Natural History of Temporal Bone Fractures

Incomplete or delayed onset of facial paralysis following a temporal bone fracture usually fully resolves (May, 1986; Fisch, 1979). Turner (1944) found that of 70 cases, 36 had an immediate palsy but overall there was a 75% full recovery rate.

Fisch (1979) felt that incomplete palsies should be treated conservatively but the mainstay of the decision to operate or not, rests on electroneuronography. This test can be used in the unconscious patient and thereby a decision as to the long term management could be decided without waiting for return of consciousness.

In general terms, longitudinal fractures with a facial palsy have a good result whereas the transverse fracture does not (Frew, 1979).

According to Fisch (1979), 20% of longitudinal fractures have a facial palsy. The fracture occurs preferentially at the tegmen tympani, labyrinth and middle ear, making the geniculate ganglion the commonest site of damage. He found that damage

usually occurs in a highly pneumatised bone and the nerve is injured by stretching along a line parallel to the tympanic segment with the greater superficial petrosal nerve.

The actual pathology present is:

(a) a bony fragment in 18%
(b) complete severance of the nerve in 29%
(c) intraneural haematoma in 53%

In 93% of these cases the lesion was immediately distal to the geniculate ganglion. Of the 28 patients in this series in 1974, 7% were in the tympanic and mastoid segment. McHugh (1959) found that 50% of his longitudinal fracture patients had the lesion immediately distal to the geniculate ganglion and were, therefore, intratympanic but probably describing the same site of lesion in a different way. This had been suggested as long ago as 1926 (Ulrich, 1926) but was ignored as confirmation was not possible without the operating microscope.

Fisch feels that the intraneural haematoma results from traction from the greater superficial petrosal nerve because of displacement of the apposing edges of the fracture. The transverse fracture is complicated in 40% of cases by a VII nerve paralysis.

Clinically there is a haemotympanum, total sensory hearing loss

and vestibular loss. Eighty per cent of these lesions lie in the labyrinthine segment and 20% in the tympanic segment (Fisch, 1979). Radiology is difficult even with tomography and CT scanning. The fracture line may be difficult to see. Bebear (1984) made the point that the fracture line is always bigger at operation than on X-ray.

Surgical Trauma

Intraoperative preservation of VII nerve function improved with the advent of the binocular operating otomicroscope initiated by Nylen from Sweden in 1921 and advanced by Shambaugh from 1940 - 1950. It was further advanced by his development of the diamond burr and suction irrigation techniques. Today's ear surgery has expanded its scope beyond the middle ear to include the middle and posterior fossa. Preoperative assessment of acoustic neuromas (Van de Heyning and Marquet, 1984) and parotid tumours (Afzelius et al, 1984) with electroneuronography may demonstrate a facial nerve at risk and hence ensures particular care intra-operatively as well as providing a reassessment of the situation preoperatively.

The incidence of surgical trauma varies. Pollman (1937) has an 0.6% incidence in 1817 mastoid operations. Zuhlke (1956) found a 1.6% incidence in first mastoid operations (1171 first operations) but for redo operations 11% (72 second operations). Miehlke (1973) feels that less than 1% of ear operations should

have a facial nerve damage. The facial nerve is particularly liable in operations in the attic filled with granulations and cholesteatoma where the nerve is not seen. Jongkees (1965) quotes 139 patients in whom a VII nerve paralysis was produced by operative intervention. In 1972 the figure had risen to 172. Mawson (1974) felt that 50% of surgical injuries in the ear occurred during a mastoid operation and Cawthorne found in 1956 that 7.5% of all facial nerve injuries were due to mastoid surgery. Shambaugh (1967) described an incidence of 0.04% of facial nerve damage in the operations of fenestration. The ultrasonic treatment of Meniere's disease puts the facial nerve at risk but the incidence reported by Frew (1979) is 0.5% of a temporary palsy and 0.0% permanent palsy. Riskaer (1946) noted an overall incidence of 2% facial nerve damage in otological cases but his operations were performed before the advent of the operating microscope between 1941 and 1943. Miehlke (1969) quotes an incidence of 1% for Germany as a whole and Palva et al (1977) quotes a 0.5% incidence for mastoid surgery.

The commonest sites of facial nerve damage are:

(a) The tympanic and mastoid segments (particularly around the oval window).

(b) The distal portion of the mastoid segment which is presumably associated with congenital abnormalities.

(c) Dehiscences of the Fallopian canal, especially above the oval window.

(d) The labyrinthine segment with an attic cholesteatoma

(e) The cerebello pontine angle with an acoustic neuroma removal.

Permeatal operations are also in the high risk category. Facial nerve damage can be caused by diffusion of local anaesthetic which is merely a temporary phenomenon and backward displacement of a wedge of bone during curattage on removal of the postero-superior meatal wall and tympanic anulus. If dissection remains lateral to the oval window, the nerve should be untouched but congenital abnormalities or obliterative otosclerosis can be misleading. Removal of osteomas with a chisel may produce a fracture line across the nerve and, therefore, it is recommended by most authorities that burrs and drills should be used in preference to gouges.

The consensus of opinion of many authors is that if the palsy is delayed or incomplete, a wait and see policy should be adopted with serial electroneuronography if possible. The vast majority of these injuries are first or second degree. Riskaer (1946) found a delayed paralysis in 29 patients after ear surgery and the majority made a good recovery. In the operation of mastoid-ectomy, the mastoid pack should be removed immediately on finding

the palsy as the pack may produce a pressure affect on the nerve or the iodine of the substance used in the pack may be neuro-toxic.

The facial nerve may be damaged in a 'too low' post auricular incision in infants. Birth trauma may be the cause and Hepner (1951) described 40 babies with idiopathic lower motor neurone facial palsy but there was no external mark of forceps or pressure. He assumed the parotid area had been squeezed by the mother's sacrum during the delivery.

Manning and Adour (1972) described 5 cases of forceps damage but these were temporary although 2 children developed mild con-tractures and synkinesis.

Avoidance is better than cure obviously and there are several guidelines which have been adopted worldwide to avoid facial nerve damage during surgery:

(a) Knowledge of anatomy.

(b) Use flat malleable retractors.

(c) Drill parallel to the nerve when taking down the facial ridge.

(d) When doing a combined approach tympanoplasty, do not rotate the drill when moving through the tympanotomy.

(e) Use diamond burrs.

(f) Use a nerve stimulator.

(g) Avoid diathermy

The facial nerve is at extreme risk in the operation of parotidectomy. Dissection on to the plane of the nerve is necessary to remove surrounding parotid tissue. The majority of injuries to the nerve in this situation are Sunderland Types 1 and 2, unless a section of nerve has had to be deliberately sacrificed in a case of malignancy.

Finding the facial nerve extra temporally may be troublesome. There are several methods of doing this:

(a) Follow the tragal pointer medially, the nerve is 1 cm medial.

(b) Bisect the angle between the anterior border of the mastoid and the vaginal process of the tympanic bone.

(c) Bisect the angle between the posterior belly of digastric and the tympanic plate.

(d) Retrace the buccal branch.

(e) Retrace the mandibular branch.

(f) Follow the posterior facial vein to the mandibular branch.

(g) Put a finger tip on the mastoid process facing anteriorly. The nerve is deep to the middle of the finger nail.

(h) Retrace the branch to the eye.

(i) Follow the tympanomastoid fissure inferiorly.

2.1 THE REPAIR OF A SEVERED FACIAL NERVE

The surgical repair of a transected nerve would ideally allow as many axons as possible to enter the distal stump to proceed to their correct end organ reinnervation. Suture of the external epineurium has been the traditional method of repair of transected peripheral nerves since the late 1800s (Orgel 1984). The choice of suture is contentious. Sunderland and Smith (1950) investigated the use of several materials on the anastomosis of peripheral nerves on the Australian opposum. The results of testing plain catgut, chromic catgut, human hair, tantalum, white silk and nylon led to the following conclusions.

(a) Some reaction is inevitable when suture materials are implanted into a nerve. This reaction is biphasic. The first reaction comprises polymorphonuclear leukocytes, lymphocytes, histiocytes, giant cells and, in the particular case of silk, plasma cells. This activity is the body's attempt to remove the foreign body. As the sutures are absorbed so the reaction subsides. The second element consists of fibroblasts which try to seal off the foreign body. The extent of this second reaction is dependent on the suture material used and is increased with non-absorbable sutures.

(b) The degree of the reaction is related to the surface area of the suture, ie the finer sutures are less reactive than

thicker sutures. There is also more reaction if longer lengths are embedded in the nerve than shorter lengths.

(c) Although the maximal reactivity area is at the suture, the reaction extends up and down the nerve for a considerable distance.

(d) The combination of the suture and tissue mass causes enlargement of the nerve at the suture site.

(e) Bundles not pierced by the suture resist invasion by the cells and fibroblasts.

(f) If small fasciculi are pierced, they obliterate but larger fasciculi do not.

(g) Nerve fibres tend to resist these reactions.

The fascicular cross sectional area of the distal stump of a severed nerve atrophied to about 40% after anastomosis compared with a normal nerve (Sunderland and Bradley, 1950a and 1950b). Thus, the reaction may contribute to distortion of axon regeneration to the original tubes with consequent loss of axons and mis-match.

The fibroblasts decrease with axon regeneration and, as the scar tissue shrinks, more new axons grow through (Weiss and Taylor,

1944). The least reactive suture material causing the fibroblastic reaction is plain catgut followed by chromic catgut, silk, human hair, and the most reactive are tantalum and nylon. The worst culprits in producing the non-fibroblastic cellular reaction are in decreasing order, tantalum, human hair, nylon, plain catgut, silk and chromic catgut. However, since this fundamental research, the suture needles have become smaller and hence excite less reaction.

Edshage (1964) compared waxed silk, silicon covered with silk, stainless steel thread, human hair, plain catgut and chromic catgut. Of these the superior suture, in terms of reaction, was stainless steel (size 7.0 to 9.0), then silk, then human hair and lastly, catgut.

Snyder et al (1968) compared silk, tantalum, collagen, surgical gut and mersilene. All produced reactions which eventally disappeared with axon regeneration. Stainless steel was the most inert but was too rigid for use. Polypropylene (Ethilon 5.0) and nylon (6.0 and 7.0) were as inert as stainless steel but free from its disadvantages. Nebel et al (1973) compared catgut, silk and homologous and autologous fibres of fascia and tendon. The autologous fibres evoked the most minimal reaction. Overall, however, they concluded that silk, nylon and stainless steel were superior to the other materials.

Plain catgut fell into early disfavour after Sargent and Greenfield (1919) described a huge early response with consequent fibrosis at the nerve suture line. At this time there was the problem of ensuring sterility and any infection at this site is disastrous. Assuming the catgut is sterile, the reaction it excites is not enough to prevent its use and it compared favourably with silk and human hair. It was not deemed suitable for fascicular repair. Forrester (1940) found catgut superior to silk in contrast to Edshage (1964) who found that catgut was so bad from his experiments that he rejected it.

Bjorkestein (1947) and Blackwood and Holmes (1954) were diametrically opposed in their findings comparing linen and silk. During World War II, tantalum was introduced as a suture material (Weiss, 1944). It was claimed that it was inert as could be demonstrated radiologically but the fact that the end of the anastomosis can separate, with no apparent radiological change, was overlooked. Sunderland and Smith (1950) demonstrated that the severity of the late reaction excluded its use at the suture line or as a protective wrapping. Spurling and Woodhall (1946) had previously claimed that tantalum was inert but these results appear to have been based on inadequate experimental studies. Woodhall (1956) and Honner et al (1970) felt that the type of suture material used had not influenced the end result or the repair of very small nerves.

Sunderland (1978) thus concluded that from all the evidence presented, the following were, in order, the most suitable for nerve repair.

(a) Silk (7.0 to 10.0)

(b) Nylon

(c) Fine stainless steel (7.0 to 9.0)

Delee et al (1977) found that, for the repair of a divided sciatic nerve in the New Zealand rabbit, prolene produced the least fibroblastic response; wire and catgut produced an intermediate reaction, Dexon the next largest reaction and silk produced the most marked reaction. All these materials were of 6.0 size. In contrast, Lee et al (1983) in a comparison of 9.0 Dexon and 9.0 nylon in the epineurial repair of the sciatic nerve that Dexon produced a lesser reaction than the nylon suture.

Most surgeons for facial nerve repair use mono-filament 10.0 sutures with a tapered needle with a diameter ranging from 70 to 140 μ. The novice is trained with 100 μ straight needle but the expert usually uses a 70 μ curved needle (Horn and Crumley, 1984).

Protective Wrapping

The concept of wrapping the anastomotic site is designed to

protect from scarring and adhesions, confine the regenerating axons to the suture line, prevent ingrowth of extramural connective tissue between the nerve ends, encourage the longitudinal alignment of components of the junction of tissue, to facilitate a smooth passage of axons across the suture line and to provide a mechanical support for the suture line to minimise tension and displacement (Sunderland, 1978).

The ideal wrap material is non-reactive and fulfills the above criteria. However, if the material is inert, it acts as a potential mechanical irritant and requires to be removed at a second operation, hence the material must be biodegradable. The following were tried during World War I - blood vessels, hens' trachea, cellophane, fascia, fat, muscle flaps, and omentum, all of which compromised the blood supply to the suture line and were abandoned. Bjorkestein (1947) however, still used a fat transplant wrap very loosely around the anastomosis to his own satisfaction. In World War II, amnioplastin and tantalum were tried but discarded (Chao et al, 1940; Rogers, 1941; Hishet and Sanders, 1943).

Millipore appeared initially to be the ideal agent as it is inert in tissues, acts as a barrier against invasion of the suture site by extramural cells, its inner surface favours normal repair and restoration of continuity at the suture line and the porosity of the membrane allows diffusion of fluids for nutrition of the suture line (Campbell et al, 1956). Millipore

is a non-woven fabric of viscous rayon coated on one side with an adhesive copolymer which is ocytacrylate and acrylic acid. Unfortunately, millipore subsequently hardens and calcifies to produce a severe tissue reaction which requires a second operation for removal. As late as 1970, McQuillan still recommended it for initial isolation of nerves before a delayed primary repair at 2 to 3 weeks.

Sialastic sheet is in vogue at present. It has been likened to a perineurial tube and is claimed to fulfill all the above requirements (Ducker, 1972). Campbell et al (1968) used a freezing technique of the nerve ends beneath the sialastic cuff because they thought this minimised funicular swelling and accumulation of fibrin between the nerve ends. They also claimed an ideal collagen framework is left for the regenerating axons if the existing ones are destroyed. This work has never been repeated. Kline and Hayes (1964) used a collagen membrane to wrap the suture line. They used a membrane of irradiated bovine flexor tendon collagen which has the advantage of absorption. The rate of absorption can be controlled by tanning. The collagen, in theory, should stimulate an immune reaction but further studies on this matter are lacking.

Midgley and Woolhouse (1968) are opposed to leaving any cuff permanently and remove their sialastic cuffs on the ninth post-operative day. Ducker (1972) claimed that, although the use of cuffs produced a better histological appearance, there was no

improvement in nerve function. The principle that any foreign material, be it collagen, millipore or sialastic, all increase connective tissue proliferation and that the functional results are inferior to simple suture was propounded by Millesi et al (1972), Sunderland's (1978) theoretical objections to wrappings are as follows: following suture the consequent oedema causes the nerve ends to swell and the cuff may become too tight, they may impair the blood supply, they may induce a foreign body reaction which may produce physical deformation of the nerve and the scarring results from fibroblasts from the epineurium, ie an internal phenomenon and not from the exterior of the nerve.

Guttman (1943) points out that little attention is paid to the handling of the nerve ends which could as easily produce the reactions described above as any suture or cuff material.

Other biological tissues used include a sleeve of a freeze dried artery (Weiss, 1943). The diameter of the vessel must be slightly smaller than the size of the nerve, hence the possibility of a compromised blood supply due to the pressure effect of the muscular coat of the vessel. Gibb (1970), therefore, recommended plasma glue to unite the ends of the nerves with a loose vein wrap around the sutured facial nerve but did not report the results.

Further weight to this suggestion was added by Hirasawa and Marmur in 1967. Braun (1964 and 1966) suggested that the best

sutureless tubulation method was by the use of a collagen tube fixed by stay sutures at some distance from the junctional zone.

Epineurial Repair

The epineurial repair has been used since the turn of the century and it remains common practice. This technique is rapid, simple, non-invasive of intraneurial contents and requires minimal magnification. The disadvantages are, however, many, ie correct fascicular opposition is compromised (Edshage, 1964) particularly with the repeated divisions and fusions of the fasciculi to form plexuses (Sunderland, 1978), simplicity encourages execution by nontrained staff, there is always some tension at the anastomosis, hence several sutures are required (both tension and extra suture material provoke fibrous tissue proliferation) and it superimposes the proliferative cut ends of the epineurium over the critical nerve gap (Terzis and Strauch, 1978).

Perineurial Repair

The description of the perineurial suture 70 years ago by Langley and Hashimoto followed on their morphological studies on the intramural organisation of the peripheral nerve trunks of the lower limbs. This technique has not advanced due to the lack of adequate magnification at operation to see the fascicles.

Optical magnification had been used for other purposes since 1923 when Holmgren developed a binocular microscope for ear surgery. In 1960, Jacobson and Suarez developed this technique to anastomose small blood vessels and subsequently recommended the use of magnification for peripheral nerve suture and the next year Smith, a plastic surgeon, introduced the technique into clinical practice (Terzis and Strauch, 1978). Despite initial suspicion, neural microsurgery flourished over conventional methods of repair but the advantages of one technique over the other remain dubious.

Millesi (1981) was a strong advocate for the interfascicular repair. He claimed this technique decreased the amount of fibrosis at the anastomotic site and left more room for regenerating axons.

At one of the commonest sites of facial nerve repair, ie the stylomastoid foramen, there is probably only a limited degree of discrete fascicle formation and often the reason for performing a graft repair may obviate fascicular repair (May, 1986a).

Indeed, Cabaud et al (1976) found no significant difference between the 2 techniques in the suture of divided cat ulnar nerves. In the human, a randomised prospective clinical study to assess these 2 techniques in the repair of severed digital nerves, failed to show a significant difference. In the digital nerve, however, there are only 3 or 4 fascicles which may allow

better alignment of the fascicles with an epineurial repair than in other larger nerves (Young et al, 1981). In contrast, Zaki and Talaat (1981) found that the fascicular repair was superior to the epineurial repair in the secondary repair of 32 median and ulner nerves of 29 patients. Bora (1976) found an increased myelin production in the posterior tibial nerve of rabbits from an immediate epineurial repair, compared with the immediate perineurial or delayed epineurial repair.

Overall, therefore, no consistent superiority of one technique over the other has been found (Wise, 1969; Grabb et al, 1970; Yamatoto, 1974; Bora et al, 1976; Cabaud, 1976 and 1980; Kline et al, 1981).

Lundborg (1988) indicated that the choice between epineurial and perineurial repair was dependent on several factors, ie the level of the injury, the relative amounts of fascicular and epineurial tissue and the timing of the surgery (primary vs secondary suture), ie perineurial repair should be used for a peripheral repair. The epineurial repair should allow orientation of the fascicles and the sutures used sparingly and left loose. Fewer sutures produce less fibrosis and a loose anastomosis allows the axons to find their distal partners more easily than if the nerve ends are squashed tightly together.

Tension at the Anastomosis

The catastrophic effect of tension is the principle culprit of either primarily or delayed proliferative connective tissue response. There is direct correlation between the length of the nerve defect, the resultant tension to achieve a direct nerve union and the fibroblastic response. Millesi and Meissl (1981) histologically linked the role of tension to connective tissue proliferation. The major source of connective tissue proliferation is the epineurium and that connective tissue invasion and intraneurial scarring took place only in the presence of tension.

Other Methods of Anastomosis

Attempts to avoid scarring from sutures and tension led to experiments in sutureless anastomosis. Sealing the nerve ends has the advantages of allowing a nearly exact coaptation, it prevents a foreign body reaction from buried sutures and reduces the surgical handling of small nerves. Artificial glueing substances, eg methyl and butyl cyanoacrylate have been tried and abandoned as they are cytotoxic and develop a progressive fibrous tissue reaction and subsequent constriction near the spots where the glue has been applied (Millesi, 1969; Berger et al, 1970).

Matras et al (1973) and Kuderna et al (1976) reported good results using the interfascicular grafting technique and a

biological non-toxic fibrinogen tissue adhesive (Tisseel). This is a 2 component system. One component is a highly concentrated human fibrinogen factor XIII, plasma fibronectin, a small amount of plasminogen and a variable amount of aprotinin. The solution is applied to the site or is first premixed with the second component which contains a bovine thrombin solution and calcium chloride. Factor XIII, a fibrin stabilising factor (FSF) activated by thrombin and in the presence of calcium, crosslinks and converts the soluble fibrin monomer gel into an insoluble polymer clot while aprotinin inhibits the fibrinolysis process induced by plasmin. According to Matras et al (1973) sealing the anastomosis with sterilised aluminium foil stimulates normal wound healing and no complications have arisen. The tensile strength of the glue clot increases as a function of the fibrinogen concentration and the polymerisation process (Factor XIII). The nerve ends of a rat were glued with concentrated fibrinogen and found to withstand a force of 1.5 neutons (Boedts and Bouckaert, 1984b) compared with the tensile strength of a single 10.2 suture which withstands 70 to 80 gm (Kuderna, 1979). In vivo fibrinolysis obviously weakens the anastomotic site and fibrinolytic activity is particularly high in inflamed and infected tissues and in prepared nerve ends and nerve grafts. The fibrinolytic activity is temporarily inhibited by local application of aprotinin. Unfortunately, high doses of aprotinin induce fibrosis. Also Factor XIII which is responsible for the crosslinking and stabilisation of the soluble fibrin gell, stimulates the fibroblastic activity.

Obviously this excess fibrinogen formation decreases the area of axon regeneration (Boedts and Bouckaert, 1984a). They report that in their rat experiments there is an important loss of tensile strength 48 hours after anastomosis despite a high dose of aprotinin and using the premixing technique with low doses of thrombin recommended by Kuderna (1979). They also used human fibrinogen which undoubtedly contributed to excess fibrin formation.

A further modification of the above technique is to combine fibrinogen glue with tubulisation using a resorbable collagen sheet of bovine origin. The tube is designed to prevent connective tissue invasion at the suture line but may also protect the nerve junctions against secondary shearing forces.

The best results were obtained using a collagen sheet with a 'rough' inner surface which gives a better gluing contact. The tensile strength of this is adequate after 48 to 72 hours. The anastomotic site is very smooth with no thickening. Boedts and Bouckaert (1984) recommend this technique for peripheral facial nerve repair.

Variations on this theme are many. Seddon and Medawar (1942) used coagulated blood plasma to unite severed nerve ends and Hoen (1946) used cockerel blood plasma and added fibrinogen and embryo tissue extract to give a stronger clot. It becomes a jelly in 1 to 2 minutes and holds the nerve ends together. He

found that there was minimal fibrous reaction and the glue was absorbed soon after union. Regenerating axons crossed into the distal stump 8 to 10 days later and advanced at a rate of 3 to 9 mm per day. The plasma clot method was further refined by Tarlov et al (1948) who used a more simple autologous plasma clot with a sleeve to compress it around the anastomosis. This produced considerably less inflammatory or fibrotic reaction and a more precise matching of the nerve ends. A further decrease in the fibrotic reaction by the use of corticosteroids was suggested by Indar and Fry (1958).

To avoid tension, Bateman (1948) used both sutures and glue with apparently good results. A variation of the plasma clot is the use of thin fibres of fibrin impregnated with thrombin and surfaced with fibrinogen. The nerve ends are apposed and the film wrapped around the junctional region until the fibrin clots. Singer (1945) claimed this had a high tensile strength with similar properties to plasma clot union.

Other methods of nerve union include the use of the carbon dioxide laser. Fischer et al (1985) felt that this method was superior in the anastomosis of the rat sciatic nerve than sutures.

Becker and Graff (1985) compared epineurial sutures with a fibrinogen adhesive technique in the rat sciatic nerve model. Neither method was better and both were inferior to the

spontaneous repair occurring after a simple nerve crush. The results were evaluated by radio labelling the metabolically active acidsoluble phosphate fractions of both nerve and muscle.

Ducker and Hayes (1968) studied the anatomical and functional results after primary nerve repair in chimpanzees. Six techniques were used and morphology, motor and sensory function were studied. Only with a thin elastic tube of silastic of 1 cm long and an internal cross-section, twice that of the nerve, was there clear consistent improvement on the morphological appearance at the repair site.

Vuursteen (1984) found that the axon population across the anastomosis decreased less with the use of a silastic cuff and indirect sutures placed at a distance from the anastomosis compared with direct sutures (86% vs 59%).

The extratemporal facial nerve is a useful model for the investigation of nerve repair and regeneration because it contains almost totally motor fibres. It is surprising that so little laboratory research has been directed at this nerve as motor pathways are commonly chosen as the model for nerve repair and the rat facial nerve is technically an easy nerve to repair.

2.2 TIMING OF NERVE REPAIR

Clinicians still debate whether an injured nerve should be repaired as soon as patient and wound status permit, or whether 2 to 3 weeks should elapse before intentionally delayed repair is carried out. Delayed repair has been favoured because of the time required to induce maximal proteosynthesis and axon sprouting (McQuarrie and Grafstein, 1973). However, immediate nerve repair is probably as effective as delayed nerve repair after facial nerve trauma (Lundborg, 1987).

The advantages of a 3 to 5 week delay before nerve suture in a contaminated field are as follows:

(a) The nerve trunk can be clearly defined from the surrounding tissue.

(b) A more accurate evaluation of the ultimate limits of intraneurial fibrosis can be made and this tissue removed.

(c) Mobilisation and transplantation procedures to bridge gaps may be undertaken.

(d) The thicker and stronger epineurium gives a more secure and reliable union.

(e) The repair can be planned and carried out in optimal conditions.

(f) The parent neurones are now in an optimal condition for regeneration (Ducker, 1972).

This latter argument is open to criticism. All parent cell bodies do not react to axon severance in the same way or to the same degree. Some show surprisingly little change while the retrograde reaction in others results in cell death. With a differential recovery rate, the onset of regeneration of the axons is dissimilar thereby invalidating an 'optimal' time for repair.

The timing of a primary suture prior to axon regeneration is not necessarily a disadvantage. Regenerative units have been seen at 36 hours post trauma and definitely within 6 days and at the time of secondary repair, prior to suture, fresh transection of the stump is necessary which may, once again, precipitate a retrograde neuronal reaction.

Following acute injury to the motor nerve fibres, there is a major reorganisation of the metabolism of the neuron. Schwann cells of the distal stump multiply reaching highest numbers at 3 days and continue to increase in number until 2 weeks following injury (McQuarrie and Grafstein, 1973).

After axonal trauma, the proximal segment increases its metabolic activity and profound changes occur in the biochemical and physiological properties of the cell body. Some of these changes are particularly appropriate to the repair process. There is a reorganisation and enhanced function of cytoplasmic RNA to a more active state directed toward reconstruction of lost axoplasm and recovery of lost connections. The materials required for transmitter function are decreased while materials required for repair of the axon are increased. By the second week following injury, the synthetic machinery rearranges and in some neurones there is a 2 fold increase in protein content. Nerve repair at this time would, therefore, in theory utilise the superior growth potential of those regenerating axons to achieve more prompt repairs.

Primary suture of a divided nerve avoids resection of the nerve ends at the secondary repair stage. No tension is involved in the repair, minimal disturbance of the funicular arrangement is made, thereby increasing the likelihood of the axons growing up the correct tube, the epineurium is thinner and more pliable and 3 to 5 weeks later and presumably allows the anastomotic area to swell more and avoid axonal damage due to constriction and it is a one stage procedure but, if a second stage is necessary, shortening of the nerve ends is avoided.

Kreutzberg and Tetzlaff (1984) studied enzymatic changes in the facial nucleus of the rat occurring after single and double

nerve transection. In a first operation the left facial nerve was 'conditioned' by transection. Then, in a second operation 2 weeks later, both right and left facial nerves were transected. Measurements of the levels and anatomic localisation in the facial nucleus of acetylcholinesterase, glucose-6-phosphate dehydrogenase, and 5' - nucleotidase confirmed that axon sprouting on the conditioned (double-injury) side was more advanced than on the single-injury side; however, the mechanisms of accelerated activity after conditioning were very complex. The authors concluded that 'a conditioning response does not simply amplify the ongoing axonal reaction and the data are in favour of a shorter initial delay' for nerve repair.

2.3 PATHOGENESIS OF SYNKINESIS AND RELATED SEQUELAE

In the human clinical situation, a major problem is the development of synkinesis or mass movements after facial nerve repair. The theoretical causes of synkinesis are as follows:

(a) imperfect regeneration due to axonal misdirection

(b) demyelination

(c) microglial scarring in facial muscles

(d) neurone depopulation

(e) multiple axon sprouting and misdirection of regeneration of axons via vertical anastomosis.

(f) disorganisation of the facial motor nucleus, consequent on peripheral nerve section (Thomander, 1984).

Radpour and Gacek (1982) studied the location of efferent neurons supplying muscles innervated by the facial nerve in the cat, by examining the facial motor nucleus after injecting horseradish peroxidase into various facial and postauricular muscles. They determined that the motor axons to the peripheral branches were located diffusely throughout the temporal course of the facial nerve. The 'mixed arrangement of motor fibres in

the facial nerve trunk accounted for the regular occurrence of a disorganised and misdirected regeneration of fibres after severe facial nerve trauma' (Radpour and Gacek, 1982).

Nerve Grafts

The closure of large nerve defects in the facial nerve has become accepted practice. Ballance and Duel (1932) report encouraging results on the facial nerve and this technique has become accepted practice (Conley, 1961 and 1988). The advantages of nerve grafting include the elimination of tension at the anastomosis, the option of placing the suture lines and the nerve grafts at a different level than the skin incision and tendon repairs, and fascicular repair if required.

The disadvantages are the need for 2 suture lines with consequent loss of axons at each end, the longer operating time and the greater likelihood of a mismatch of axons across the anastomosis.

Stevens et al (1985) found that the longer the nerve graft in experimental rats, the poorer the result. Presumably this was due to extensive mobilisation of the nerve with consequent embarrassment of its blood supply.

Ellis and McCaffrey (1985) used 100 rats in an experiment to differentiate by electrophysiological means whether the functional

results were superior if the repair was primary or delayed. Five groups were used: Group 1 represented the use of fresh grafts in fresh defects, Group 2 represented the use of predegenerated grafts in delayed defects, Group 3 represented the use of predegenerated grafts in fresh defects, Group 4 represented the use of fresh grafts in delayed lesions and Group 5 were controls. There was no significant difference between immediate or delayed nerve repair or between fresh or predegenerated nerve grafts.

Gordon et al (1979) described the effect of grafting fresh and 2 week old defects of the rat femoral nerve. They tested regeneration 40 days following grafting by measuring twitch strength of muscle by stimulating the reconstructed nerves and also by determining axon counts proximal and distal to the graft. They obtainedthe greatest number of distal axons when predegenerated grafts were placed in precut (delayed nerve) defects and obtained only slightly poorer results than when fresh grafts were placed in fresh defects. The results of placing fresh grafts in precut (delayed) defects or predegenerated grafts in fresh defects were significantly poorer although some of the results of the study were incomplete which makes the results difficult to interpret.

Carcinoma of the parotid gland accounts for about 10% of head and neck malignancies (Robinson-Baker et al, 1966). Eighteen per cent of these author's series and 12% of Conley and Hamaker's (1975) series of parotid carcinoma presented with some

degree of a facial palsy. Those patients with facial nerve involvement seem to have a less favourable prognosis than those in which the facial nerve was intact. Thus, in patients with facial nerve palsy, Conley and Hamaker (1975) report a 26% 5 year survival and Eneroth (1972) had no 5 year survivals.

The poor prognosis and the severe cosmetic deformity resulting from sacrifice of the facial nerve mitigated against radical surgery but Conley required to remove all or some of the facial nerve in 68% of his 279 patients with parotid carcinoma. The usual method of rehabilitation is the insertion of an autogenous nerve graft to bridge the gap.

Lathrop (1963) and Afzelius (1984) reported results that post-operative radiotherapy had a markedly detrimental effect on the return of facial function after grafting. McGuirt and McCabe (1977) in contrast in the study of facial nerve autografts in cats, found that postoperative radiotherapy had no effect on the outcome of facial nerve function. Stearns (1982), again in contrast, found preoperative radiotherapy made a significant difference in nerve regeneration in the sciatic nerve of rats in which a 5 mm section of nerve was excised then replaced and sutured. He found that the epineurium of the irradiated nerves was thicker and surrounded by more adhesions than the normal irradiated nerve.

Pillsbury and Fisch (1980) reported the facial reanimation

results of 19 patients, 9 of whom had had postoperative radiotherapy and 2 had preoperative radiotherapy, who had a facial nerve graft involving all divisions. They were assessed at least one year postoperatively by standard photographs. Radiotherapy reduced the average postoperative results from 70% to 25% of normal movement.

Speiss (1980) irradiated the sciatic nerves of rats with the equivalent of a standard human dose of 3 - 8000 rads. After 6 months, normal conduction times with no apparent functional impairment was noted but histology showed axonal degeneration and fibrosis of the endothelium of the capillaries in the myelin sheath. This relative ischaemia may assume greater influence on regenerating axons through an anastomosis which is normally relatively avascular. There is, of course, a considerable difference between animal and human nerves in regenerating potentials, differentiation of movements and size.

2.4 TRANSPOSITIONS

The most popular transposition operation is the hypoglossal facial 'hook-up'. The hypoglossal nerve is dissected out as distal as possible and the facial nerve sectioned as it exits the stylomastoid foramen. The hypoglossal nerve is larger than the facial nerve and, therefore, the latter is sectioned obliquely to obtain a good, neat anastomosis. Fisch (1979) thinks this oblique section is superior to allow the maximal area for neurone growth but Lathrop (1963) not only does not believe this but he also does not think the epineurium contributes to scarring and, therefore, cuts it in a straight section.

Conley and Baker (1979) reported on 137 cases of this procedure. Twenty two per cent had minimal atrophy of the tongue, 53% had moderate atrophy of the tongue and 25% had severe atrophy of the tongue. Three per cent complained about abnormal chewing movements, 2% on swallowing difficulties, and 2% on speech difficulties. Ninety five per cent of their patients developed some type of quality of movement and 15% complained of too much movement, particularly when chewing.

Splitting the hypoglossal nerve and using the descendans hypoglossi gives very poor results and, according to Crumley (1984d), should be abandoned.

In 1895, Sir Charles Ballance anastomosed the facial nerve to the

accessory nerve, end to side (Ballance, 1920). The end result was not known but Cushing (1903) reported a similar case which produced facial symmetry, reasonable eye closure and a level mouth. The results of nerve anastomosis remained unclear (Ballance and Duel, 1923/24) and Ballance and Duel (1932) claim little more than improved tone of the facial muscles after a facial glossopharyngeal anastomosis.

Scaramella in 1970 presented a patient in whom the intact buccal ramus on the non-paralysed side was anastomosed to the paralysed stem of the facial nerve with a sural nerve graft. The technique has been expanded and developed by Anderl and Samii (1977). The length of the graft is 6 to 18 cm and interfascicular repair performed. Anderl favoured a 2 stage procedure to allow axons to grow to the opposite side, he then resected the consequent neuroma at the distal end of the graft and histologically confirmed the presence of axon sprouting before completing the procedure. In contrast, Anderl (1973) and Samii (1975) repaired both sides at once and claim good results. There is disagreement over reversal of the graft or not. In theory, if the graft is reversed, all the axons will grow to the end but if it is not reversed then the peripheral divisions allow axons to reinnervate all the muscles en route. The timing of the second stage is usually at about 6 weeks.

There are, therefore, many possible combinations of nerve grafting and reinnervation of the facial nerve other than a

straightforward nerve interposition. On occasion there are reports of spontaneous return of movement despite the fact that a nerve has been cut and there is quite a gap between the ends. Conley (1955) reported 2 such cases and postulated that trigeminal nerve function takes over the facial function. Helsper and Martin (1957) felt that 25% of his patients had some spontaneous return of function. Nishimura et al (1977) demonstrated that the facial nerve crosses the midline by several centimetres and could possibly be the source of regenerating axons.

If the function has not recovered by 18 months, it is unlikely to recover at all. This is probably because of degenerating motor end plates. This degeneration is exponential and there is no end stage fibrosis of these motor end plates (Conley, 1984). Although 18 months is the upper limit of time to wait to ensure a good result because of irreversible axonal changes by this time. The most reasonable approach seems to be by Crumley (1984b) who feels that grafting can be a viable proposition up to 2 years but, thereafter, there are too few motor end plates to make an appreciable difference. Before 18 months there are always enough motor end plates to allow a reasonable return to function. The difficult time is 18 to 24 months and Crumley depends on the electromyographic activity in this period.

2.5 SURGICAL ANATOMY OF THE RAT FACIAL NERVE

Animal models of facial nerve healing and regeneration are becoming increasingly important as new techniques of facial nerve repair and grafting are developed. Mattox and Felix (1987) have described in detail the peripheral branching pattern of the rat facial nerve and performed axon counts on the main trunk and peripheral branches. Similarities and differences between the human and rat facial nerve are emphasised and the branches of the rat nerve amenable to experimental manipulation are identified. In addition, electrophysiologic data from the rat show a more diffuse pattern of innervation of the face from the individual branches than seen in humans.

There is no ideal animal model for clinically related studies of the facial nerve. The models that have been used most extensively include the cat (Yamamoto and Fisch, 1975; McGuirt and McCabe, 1977), rabbit (Corte et al, 1984) and guinea pig (Buch, 1970) facial nerves. Other clinically related studies of nerve suture, grafting, and radiation effects have used sciatic nerves of the rat (Ellis and McCaffrey, 1984) and rabbit (Orgel and Terzis, 1977). Each of these models has its own advantages and disadvantages. Large animals are expensive and reliable anaesthesia may be a problem. A long segment of the rat sciatic nerve is easy to expose, however, this nerve is already separated into distinct abductor and adductor fascicles when it emerges from beneath the sacrum. Therefore, there could be

profound alteration in the functional result of an anastomosis or graft, depending upon the alignment of the proximal and distal segments.

Mattox and Felix (1987) investigated the rat facial nerve as a potential experimental model. However, details of the rat facial nerve anatomy in published texts are contradictory, (Green, 1985; Hebel and Stromberg, 1976) and certain aspects were not in accordance with their observations. They dissected out the facial nerves of 10 rats under microscopic control and found that in the rat the facial nerve exits the stylomastoid foramen on the lateral side of the skull, posterosuperior to the external auditory canal. The posterior auricular branch diverges from the facial nerve as it leaves the stylomastoid foramen. It then passes anteroinferiorly between the trapezius muscle and the external auditory canal, covered by the cervical head of the trapezius muscle for the first 2 mm. The common trunk of the occipital and postauricular arteries passes beneath the mid portion of the main trunk on its way to the pinna. The artery is separated from the nerve by a thin fascia. The total length of the main trunk in situ is 6 mm.

Six millimetres from its exit from the stylomastoid foramen, the nerve divides into 6 peripheral branches. Mattox and Felix (1987) found only 1 - 2 mm length of the discrete tempero, facial and cervical facial divisions previously described (Green, 1955; Hebel and Stromberg, 1976).

All the peripheral branches lie beneath the superficial fascia covering the facial muscles and the nerve passes beneath, not within the parotid gland.

Branches

(a) Posterior Auricular Branch: The auricular branch diverges from the facial nerve as it leaves the stylomastoid foramen and supplies the auricular musculature.

(b) Posterior Cervical: A small posterior cervical branch was the first branch of the furcation. This branch was the only one to consistently pass lateral to the external jugular vein.

(c) Cervical Branch: The cervical branch is the most posterior and inferior branch beneath the external jugular vein. It separates immediately from the mandibular branch not midway across the masseter muscle as previously described.

(d) Mandibular Branch: The mandibular is the most obvious branch of the facial nerve. It is readily identified as it crosses the lower central portion of the masseter muscle. Macroscopically, the mandibular branch extends for 10 - 12 mm before it has diverging branches. Peripherally, fibres from the mandibular nerve can be traced to both the lower and upper lips.

(e) Buccal Branch: The buccal branch follows the groove between the masseter and the temporalis muscle. It passes beneath the eye toward the nose, however, peripherally it sends branches to both the upper and lower lips.

A branch of the auriculotemporal branch of the trigeminal nerve passes around the posterior edge of the mandible and joins the buccal branch 2 - 4 mm distal to the furcation. This nerve closely follows the facial nerve but can be dissected from it with the operating microscope. Peripherally, the trigeminal fibres go to the skin in the area of the whiskers.

(f) Temporal and Zygomatic Branches: The temporal and zygomatic branches may take off from the furcation of the main trunk or may branch off the buccal branch. They have short, complex branching patterns. They are difficult to expose because of their position near the eye and their intertwining with the peripheral branches of the external jugular vein.

The axon counts of the buccal division of the facial nerve showed an average of 1955 axons. Care was taken to avoid counting the axons of the trigeminal divisions.

Attempts at direct nerve conduction studies were fraught with problems and an action potential of any consequence was obtained

in a small minority of the rat facial nerves. The lack of reproducibility of this technique renders it practically worthless.

The remainder of this thesis describes several experiments on rats to determine, in principle, the best method to anastomose a divided peripheral branch of the facial nerve.

In the human, the final assessment of a satisfactory repair is the subjective opinion of the patient of what he can do compared to what he was able to do before surgery but this does not provide any objective criteria for use elsewhere. Laboratory studies never reflect the success of either the nerve surgery or of the techniques used for repair but do allow precise controls to be applied and hence yield data which can be interpreted statistically. They are, therefore, invaluable in defining aspects of this technique which should or should not be applied where controlled conditions do not exist.

3. ESTABLISHMENT OF EXPERIMENTAL METHODS AND CONTROLS

The restoration of facial movement and facial symmetry after resection of the facial nerve during parotid surgery for cancer is of paramount importance to the patient. The actual technique of grafting the facial nerve remains debatable and, although an anastomosis of peripheral nerves is commonplace, few comparative studies have been described on the facial nerve. The usual site of anastomosis after extirpation of a malignant parotid tumour is in the extratemporal portion and may involve the common trunk or any one of the peripheral branches. This study uses the rat as an experimental model and compares, in principle, differing types of anastomostic agents to rejoin the divided ends of the buccal division of the facial nerve. In this chapter the agents are tested and compared to controls to avoid any experimental bias resulting from the materials used.

3.1 METHODS AND MATERIALS FOR TRAINING PURPOSES

Twenty Sprague-Dawley rats were used for training purposes. These rats were either dead or decerebrate. The buccal division of the rat facial nerve is a small nerve of a diameter up to 1 mm. Although an otological surgeon (the author) uses the microscope on a daily basis, handling a nerve of this size requires a particular expertise. To gain this expertise, a training period was used. The approach to the nerve was standardised for this technique and the other experiments

subsequently discussed, with the exception of the experiments on the sciatic nerve, used the same technique. The fur overlying the buccal division of the facial nerve was trimmed and a 2 cm horizontal incision was made from a point equidistant from the eye and the angle of the mouth running towards the snout. The nerve is found under the deep fascia, closely applied to the masseter muscle. The nerve emerges from the undersurface of the parotid gland which is better defined in rats than in humans. The nerve was identified from the parotid duct which is in close relationship. Sharp dissection separated the nerve atraumatically from the underlying muscle for a distance of 2 cm. A sharp scalpel was used to divide the nerve transversely. Two epineurial sutures of 10/0 Ethilon were placed under microscopic control superiorly and inferiorly to anastomose the nerve. The result was critically assessed and repeated until it was acceptable and the experimenter felt confident that his surgical technique was sound. The contralateral nerve was similarly treated. A total of 40 facial nerves were anastomosed. All operations were performed by the author.

3.2 METHODS AND MATERIALS FOR CONTROL EXPERIMENTS

Live, healthy Sprague-Dawley rats of 6 - 8 weeks old, weighing between 200 and 300 gm were used as the experimental model. All experiments were carried out in the research laboratories of Ethicon Limited, Bankhead Avenue, Edinburgh, EH11 4HE, under Animal Licence No PIL 60/01734. The rats were individually anaesthetised by an intraperitoneal injection of Midazolam in a doze of 0.1 mg per 100 gm of body weight. Midazolam is a water soluble benzodiazepine and was chosen to avoid any neuromuscular action. The rats were prepared as detailed above and all the operations performed under impeccable sterile conditions. The left side was always the first side to be operated on. The nerve was isolated for a distance of 2 cm and a sheet of plastic was inserted deep to the nerve to separate it from the underlying muscle to highlight it. The nerve was cleared of any adherent excess soft tissue. A Digitimer Limited isolated stimulator model DS2 was used as a source of electrical energy. Two hooked electrodes were inserted under the nerve and the nerve was gently lifted clear of the underlying plastic sheet. These were connected to the electrical stimulator. The voltage of the impulse from the stimulator was gradually increased until the muscles of the face could be seen to move minimally. Care was taken to allow an interval of several seconds between impulses to avoid summation. Detection of the movement was facilitated by inserting a coloured pin in the snout of the rat. Any minimal movement of the snout was increased due to the

length of the pin. The voltage of minimal stimulation was noted and repeated. The mean of 3 readings was taken as indicative of the voltage required to achieve a minimal stimulation. The voltage of the impulse was then increased until a maximum excursion of the pin was noted. The voltage used to just produce the movement was noted. The recordings were repeated on 3 separate occasions and the mean taken. The nerve was then sectioned in a transverse fashion, using a fresh scapel blade.

In some of the control experiments both the nerves were left intact as detailed below. The sectioned nerve was anastomosed as detailed below. The end result was photographed through a microscopic arrangement. This magnified the nerve 10 times. Each photograph was taken with the same camera and the magnifying lens and the microscope was the same throughout all the experiments. All the nerves were photographed in focus. This ensured that the distance between the nerve and the camera was the same and allows a comparison to be made of the width of each nerve. A fixed distance from microscope lens to operating table was not used because this would not have taken into consideration the varying sizes of rats' heads. A 6/0 prolene marker suture was placed in nearby muscle to mark the anastomotic site. The plastic strip was removed and the wound sutured using 6/0 prolene interrupted sutures. The animal was then left to recover. If the animal scratched out at the wound and the sutures came out, they were replaced as necessary. The animals were kept in cages for 70 days, no more than 2 to a

cage. At 70 days the animals were reanaesthetised and prepared as detailed above. The prolene marker suture was identified and anastomotic site identified. The nerve was dissected free of the underlying muscle. A plastic strip was inserted deep to the nerve separating it from the underlying muscle. The anastomotic site was photographed as detailed above. The voltage required to produce a minimal and maximal twitch was noted in an identical manner as detailed above. The nerve was then divided at 1 cm proximal and 1 cm distal to the anastomosis. The section was placed and pinned onto cardboard marked proximal and distal appropriately. The animal was then killed with an over-dose of thiopentone. The nerve specimen was placed in 10% buffered formalin to completely fix it for 24 hours. The nerve was then sectioned at 3 mm either side of the anastomosis. Each piece of nerve was uniquely identified and processed through the standard Ethicol Glycol Methacrylate procedure.

The cut face of the nerve was orientated downwards in the Meth-acrylate block. After the tissue was processed, sections were cut at 3 micrometres. The sections were rinsed in distilled water, briefly rinsed in 70% industrial methylated spirits and placed in a saturated Sudan black B solution in 70% alcohol for 45 minutes at room temperature, rinsed briefly in 70% industrial methylated spirits then in distilled water and mounted in glycerine jelly on a glass slide (Appendix 3.1). The sections were then viewed using a compound microscope (Jemaned, Karl Zeiss, Jena) connected to a video 3 semi-automated image

analysis system (Analytical Measuring Systems Limited, Pampisford, Cambridge). Distribution histograms of myelinated nerve fibre diameters were compiled from the sections using a Magiscan M2A Automated Image Analysis system (Joyce-Loebl Limited, Gateshead, Cleveland, UK). Artifact rejection was set to eliminate fibres in the 0.75 range of the function (4 Pi.A) over P^2 where Pi = 3.14286, A = cross sectional area of the axon in micron^2 and P = perimeter (microns). This effectively excludes fibres not cut in transverse section and those showing excessive crenation due to poor fixation. Fibre size distributions were compared using Ogival (cumulated frequency) curves (Appendix 3.3). In all experiments the sides of anastomotic material were randomised and the assessments were performed blind.

Five rats had a 10/0 Ethilon suture placed through the intact buccal nerve on one side and a 10/0 Vicryl suture placed through the contralateral intact buccal nerve (Fig 3.1). Five rats had Tisseel glue placed alongside the intact buccal nerve on one side and a collagen tube wrapped around the contralateral intact buccal nerve. Ethilon is a non-absorbable monofilament suture and the sutures used were code W2830, batch BTJIEX of size 10/0 (75 μ), sample no 8745. Vicryl is an absorbable braided suture and the sutures used were code W971G, batch 301181 of size 10/0 (75 μ), sample no 8746. Both these sutures are manufactured by Ethicon Limited (Fig 3.2). The glue used in all the experiments was Tisseel kit which is a human fibrin seal/bovine thrombin and

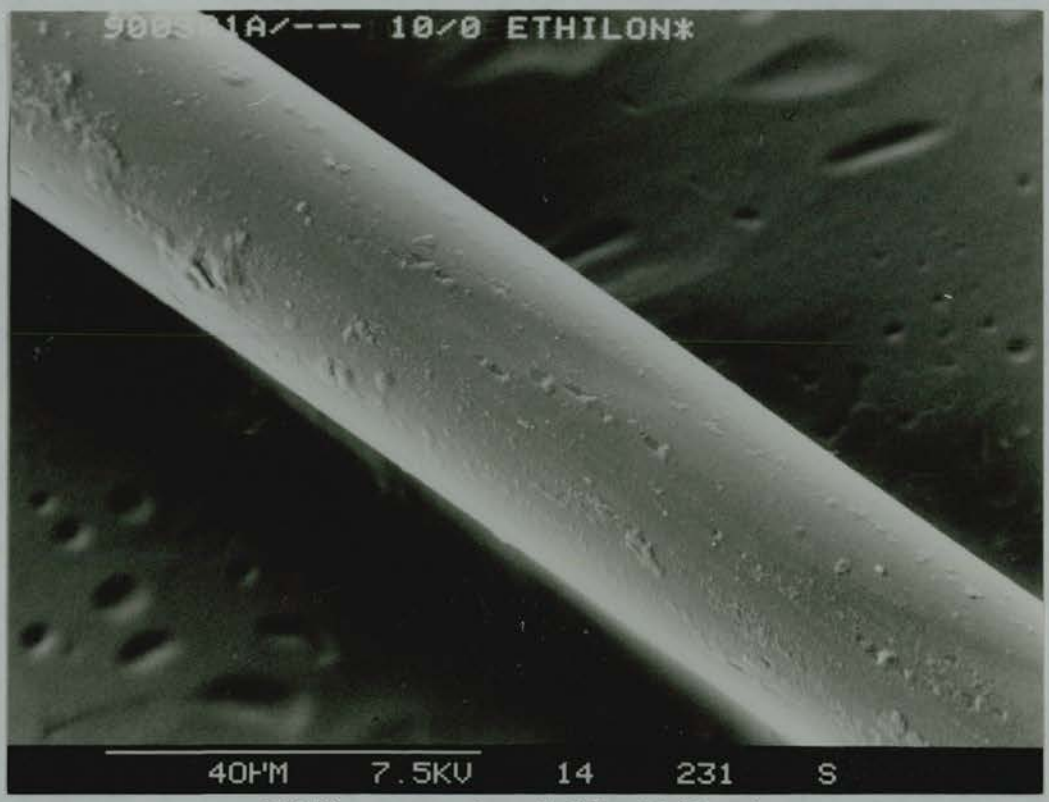
FIGURE 3.1 - THE BUCCAL DIVISION OF THE RAT FACIAL NERVE

Intact

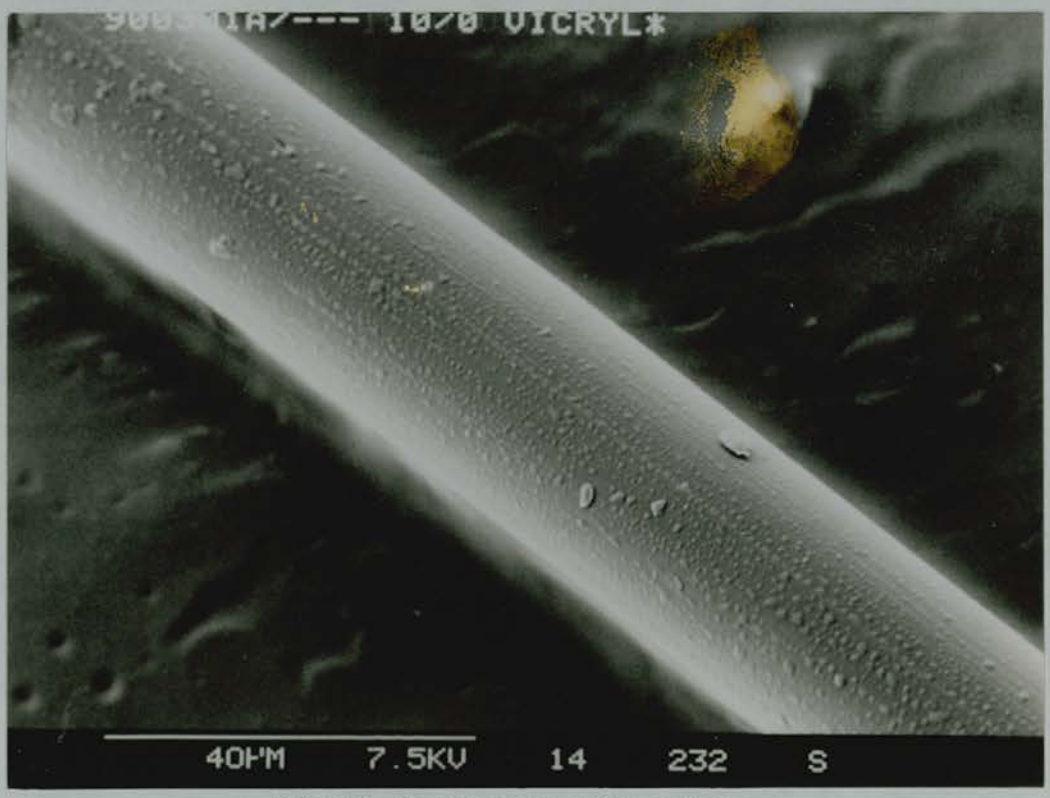
Divided

Sutured

FIGURE 3.2 - SCANNING ELECTRON MICROSCOPY OF THE SUTURES USED



Ethilon non-absorbable 10/0 suture



Vicryl absorbable 10/0 suture

the batch no used was P21 061410178704T, sample 9496. The collagen tube used was a 0.3% collagen film wrapped around the nerve manufactured by Devro, sample no 9498.

Five rats had the nerve cut on one side, the ends placed together but no anastomotic agent used. On the contralateral side, the nerve was cut and reanastomosed using Ethilon 10/0 sutures as detailed above. Five other rats had the same procedure but the Ethilon sutures were substituted by Vicryl. Five further rats had the same procedure but substituted Tisseel glue for the Ethilon and Vicryl. Lastly, 5 separate rats had the same procedure but the collagen tube substituted for the anastomotic agent (Table 3.1).

The collagen film had previously been tested by personnel of the research laboratory of Ethicon Ltd. A total of 6 Sprague-Dawley rats were taken and under general anaesthesia, 2 samples of the collagen (10 mm x 0.25 mm) were inserted into 2 separate pouches of the lumbar muscle. Two rats were killed at 28 days, 2 at 49 days and 2 at 70 days. Even by 28 days there was no convincing evidence of collagen film or of tissue reaction and by 70 days there was total absorption of collagen film (Appendix 3.2).

Thirty live Sprague-Dawley rats were used for this control experiment. These were 7 to 8 weeks old and weighed between 200 and 300 gm. The rationale of photographing the nerve before it was divided and again at postmortem was to ensure a degree of

quality control. The ratio of the transverse diameter of the nerve 1 cm proximal and 1 cm distal to the anastomosis was taken as an indication of any natural change in the diameter of the nerve. The same measurements at postmortem were expressed as a ratio and this latter ratio divided by the former ratio to determine any real change in the diameter of the nerve.

3.3 RESULTS

Photography

There was no significant difference in the size of the transverse diameter of the nerve of either side of the rat preoperatively and postoperatively in any of the rats used in this experiment. After the training period, the facilitation of handling the sutures or the tube and the glue were all similarly straightforward and there appeared to be no difference in the immediate result of the anastomosis. A qualitative assessment of the post-mortem photographs was made and they were ranked in order of quality of repair and nearness of normality to the nerve. The Wilcoxon Rank sum test was used but no significant difference was found between any of the groups described above (Table 3.2). There was no macroscopic evidence of vicryl glue or collagen tube at post-mortem

Electrophysiology

The results are summarised in Table 3.3. The only significant result is that of a p value of less than 0.05 in the minimal nerve test comparing the before and after results when using glue. This is probably artifactual as it is not supported by the result from the maximal nerve test.

Histology

Both the proximal and distal nerve sections were studied and one can see from Table 3.4 there appears to be no difference in the histological appearance whether Ethylon or Vicryl, or the tube or the glue is laid next to the nerve or through the nerve (Table 3.4 and Table 3.5, Figs 3.3 - 3.11).

Scanning electron microscopy showed no qualitative difference in any of these nerves (Fig 3.12a and Fig 3.12b, Appendix 4.1)

It would appear, therefore, that there is no difference and no effect on the nerve by these anastomotic materials.

Discussion

These various anastomotic agents for nerve repair have been common place for centuries. Throughout medical literature, however, there is a conspicuous lack of understanding of the effects of those agents on neural tissue per se. In any experiment it is crucial to use controls to ensure that the results obtained are a direct result of the experiment and not a reflection of other factors. In the experiment described above, which is to study the effect of the materials used to anastomose the buccal division of the rat facial nerve, the controls were as follows. A single operator trained himself to perform the anastomosis to a standardised acceptable standard, the various

FIGURE 3.3

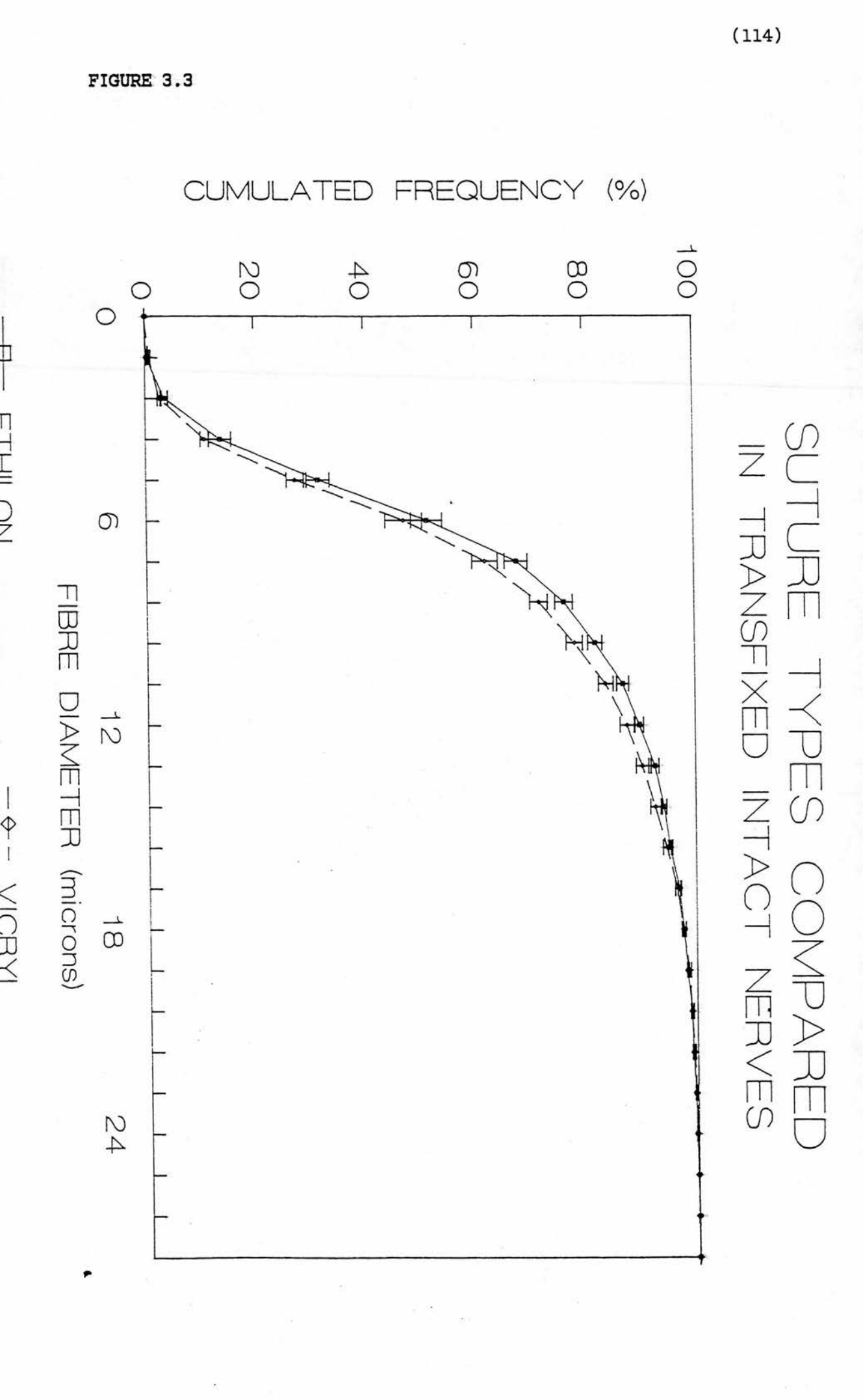

SUTURE TYPES COMPARED
IN TRANSFIXED INTACT NERVES
CUMULATED FREQUENCY (%)
0
20
40
60
80
100
0
6
12
18
24
FIBRE DIAMETER (microns)
ETHILON
VICRYL

FIGURE 3.4

SUTURE TYPES COMPARED
IN TRANSFIXED INTACT NERVES
PROXIMAL
N=5 RATS
DISTAL
N=5 RATS
MEAN FIBRE DIAMETER (microns)
0
1
2
3
4
5
6
7
8
9
10
VICRYL
ETHILON
TYPE OF TRANSFIXING SUTURE

FIGURE 3.5

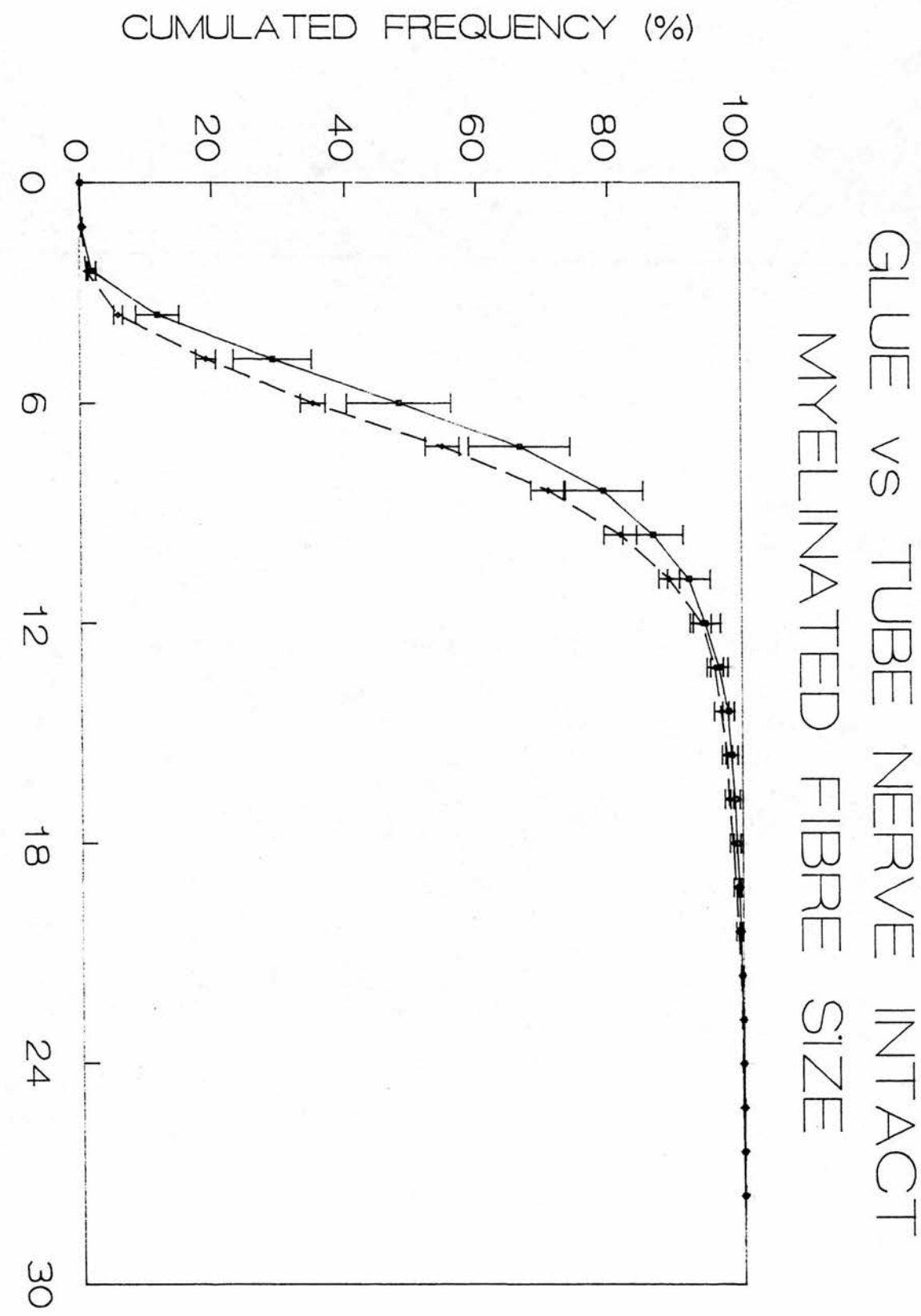

GLUE vs TUBE NERVE INTACT
MYELINATED FIBRE SIZE
CUMULATED FREQUENCY (%)
0
20
40
60
80
100
0
6
12
18
24
30
FIBRE DIAMETER (microns)

FIGURE 3.6

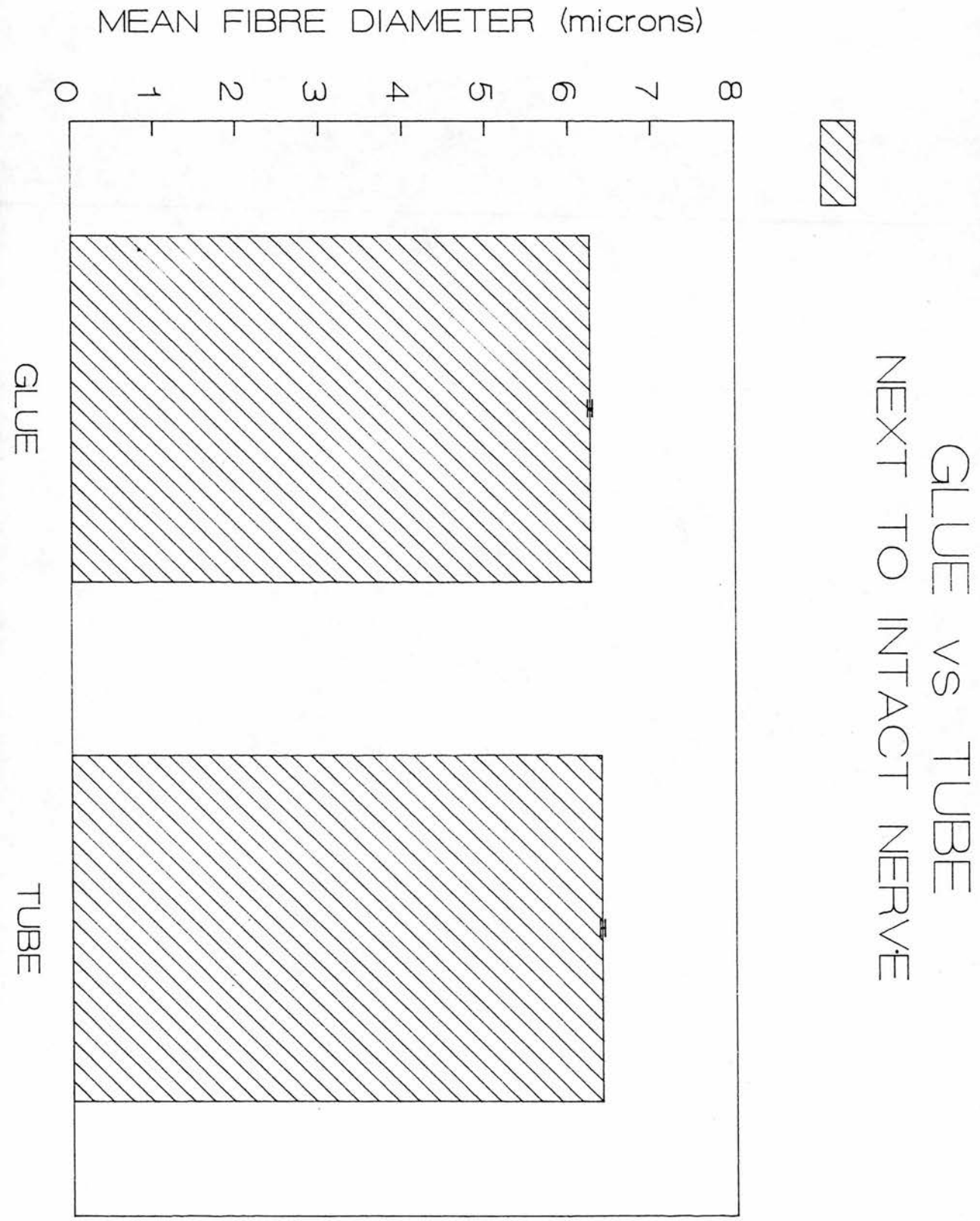

GLUE VS TUBE
NEXT TO INTACT NERVE
MEAN FIBRE DIAMETER (microns)
0
1
2
3
4
5
6
7
8
GLUE
TUBE

FIGURE 3.7

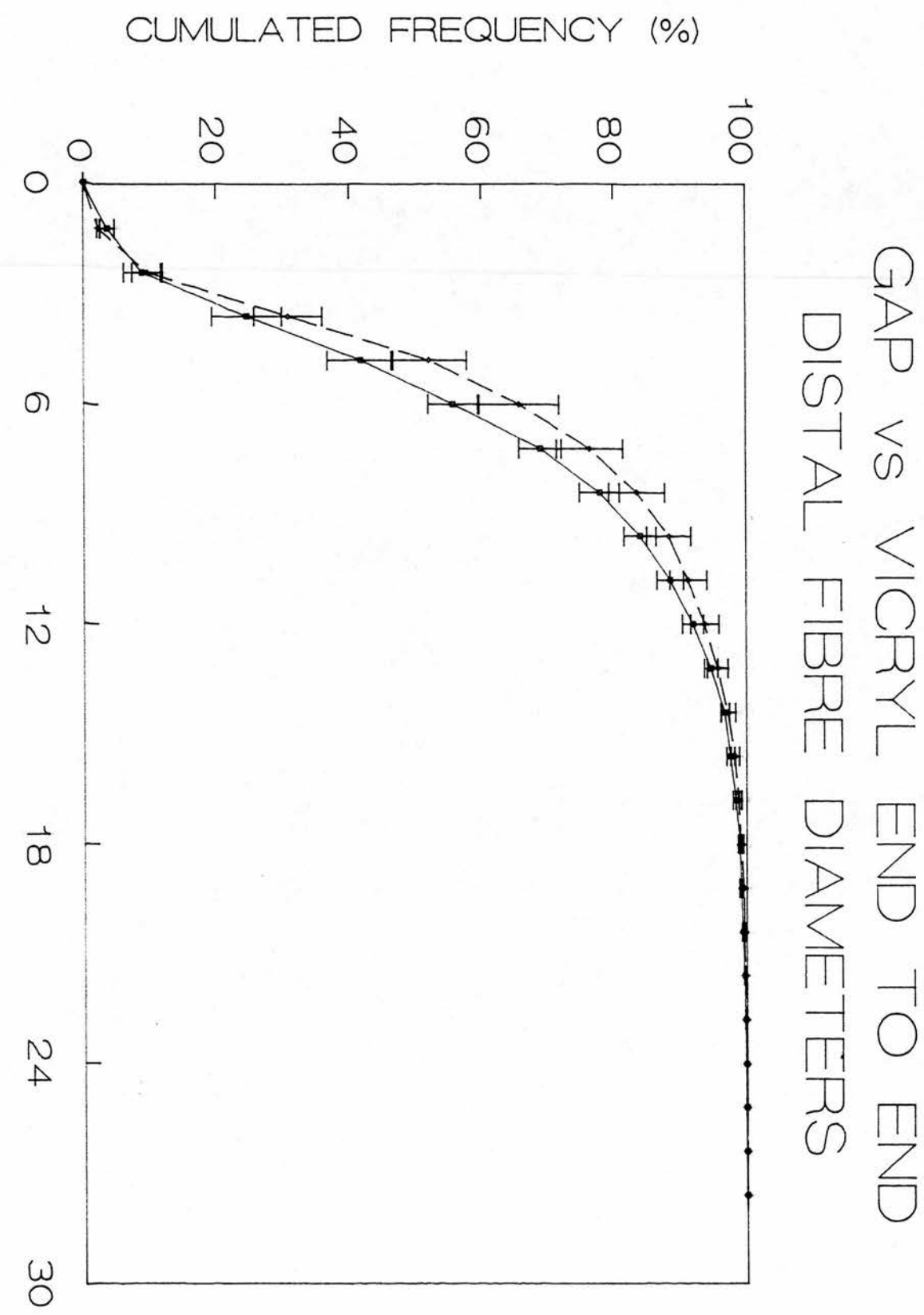

GAP VS VICRYL END TO END
DISTAL FIBRE DIAMETERS
CUMULATED FREQUENCY (%)
0
20
40
60
80
100
0
6
12
18
24
30
FIBRE DIAMETER (microns)

FIGURE 3.8

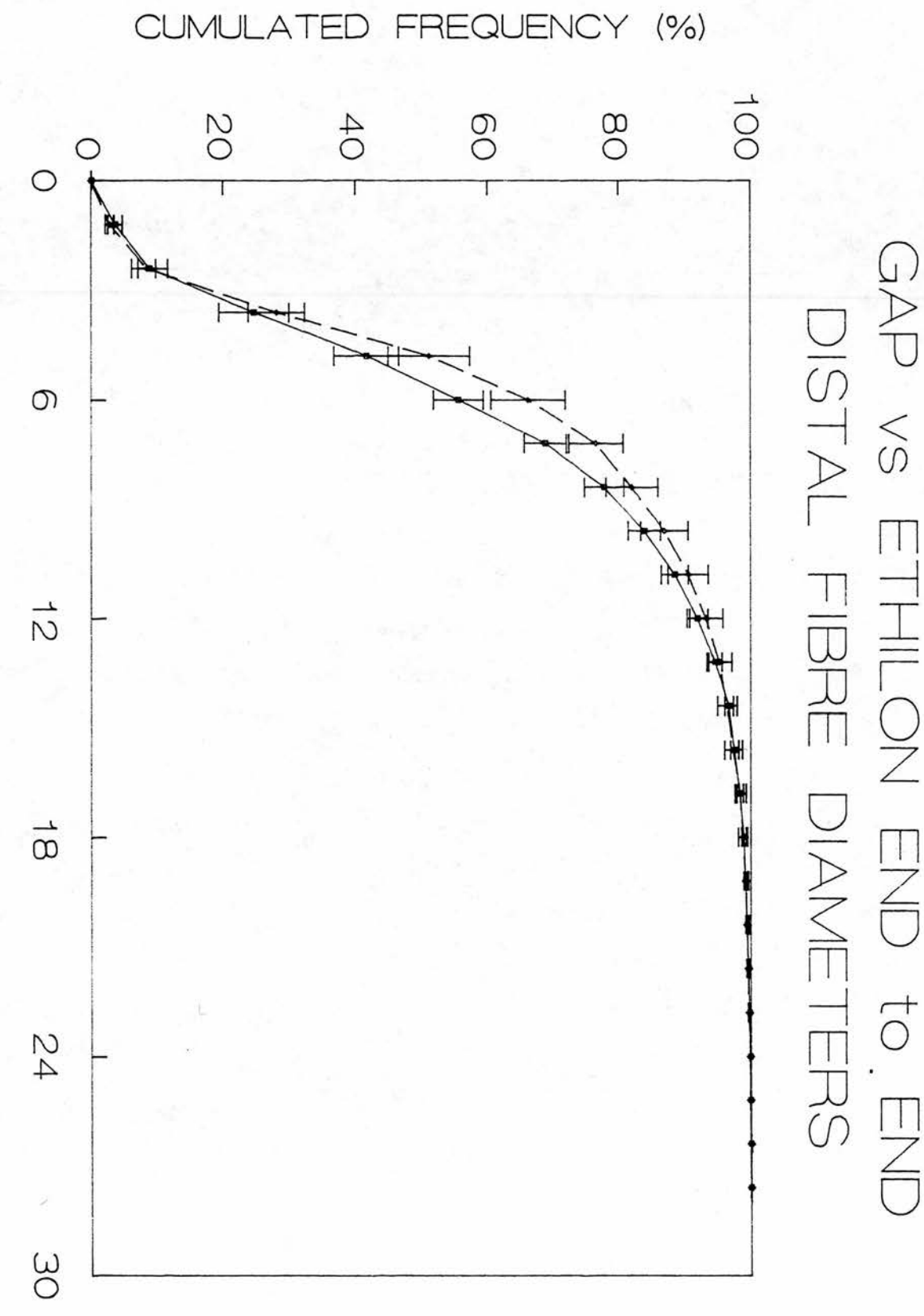

GAP vs ETHILON END to END
DISTAL FIBRE DIAMETERS
CUMULATED FREQUENCY (%)
0
20
40
60
80
100
0
6
12
18
24
30
FIBRE DIAMETER (microns)

FIGURE 3.9

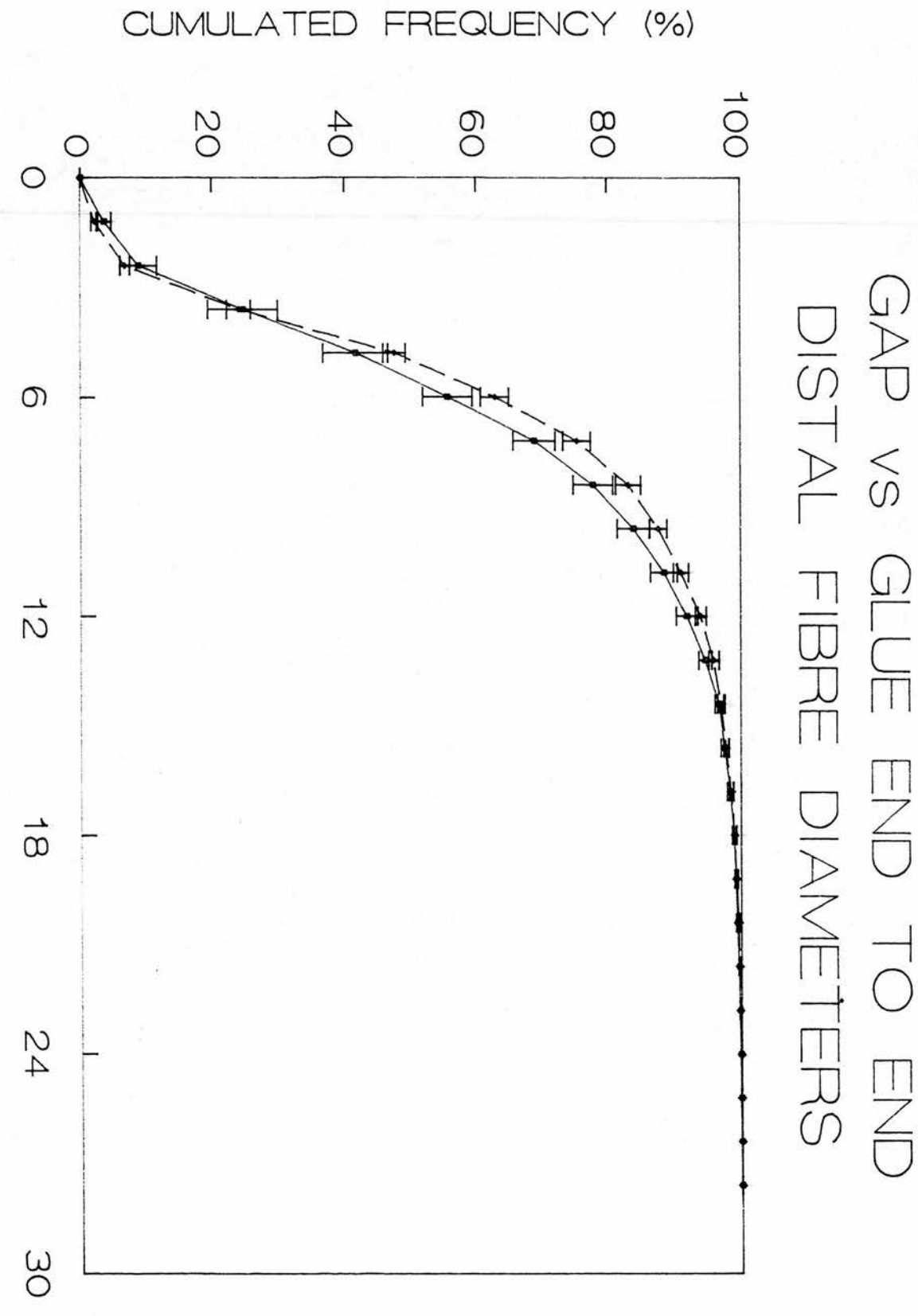

GAP VS GLUE END TO END
DISTAL FIBRE DIAMETERS
CUMULATED FREQUENCY (%)
0
20
40
60
80
100
0
6
12
18
24
30
FIBRE DIAMETER (microns)

FIGURE 3.10

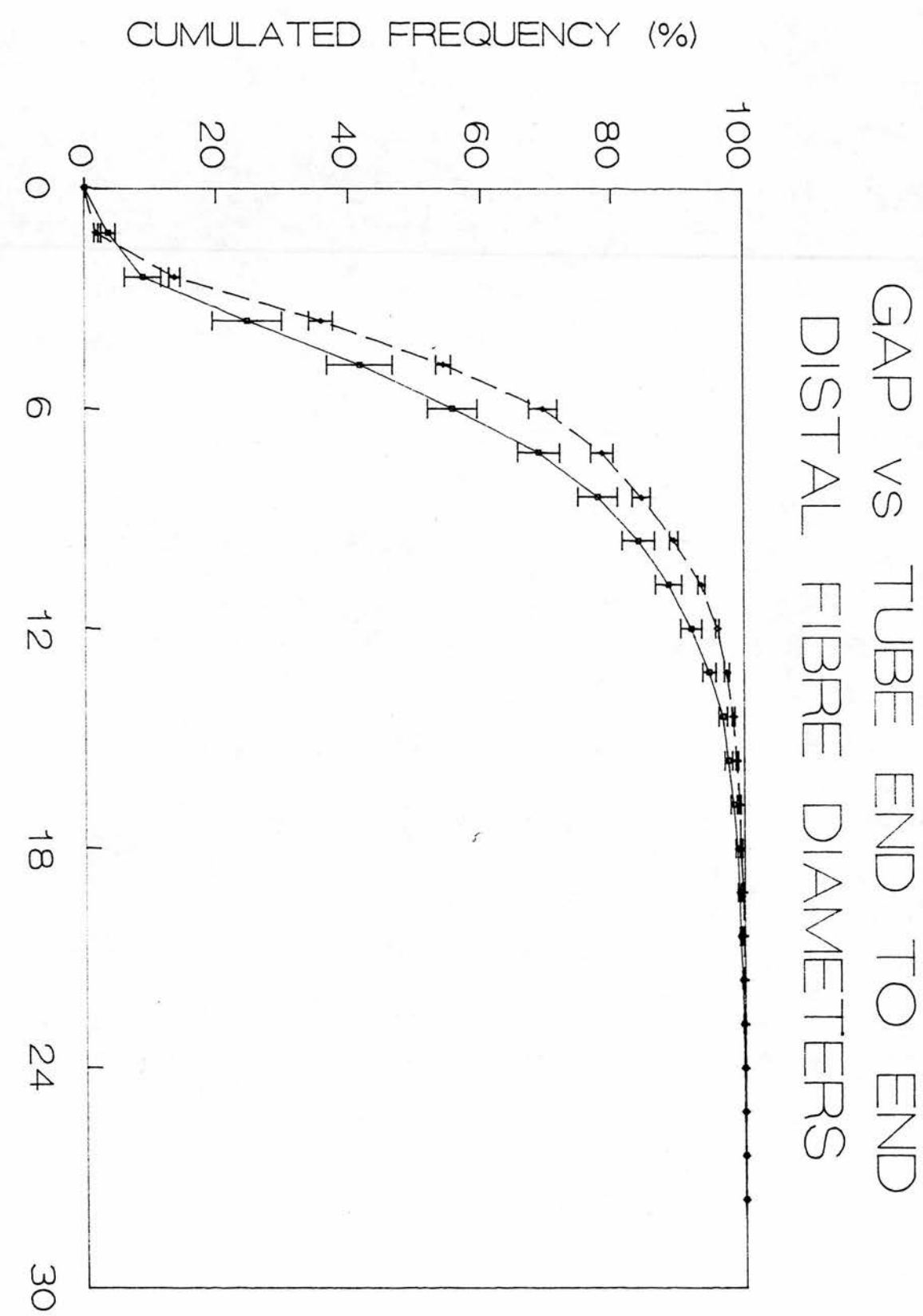
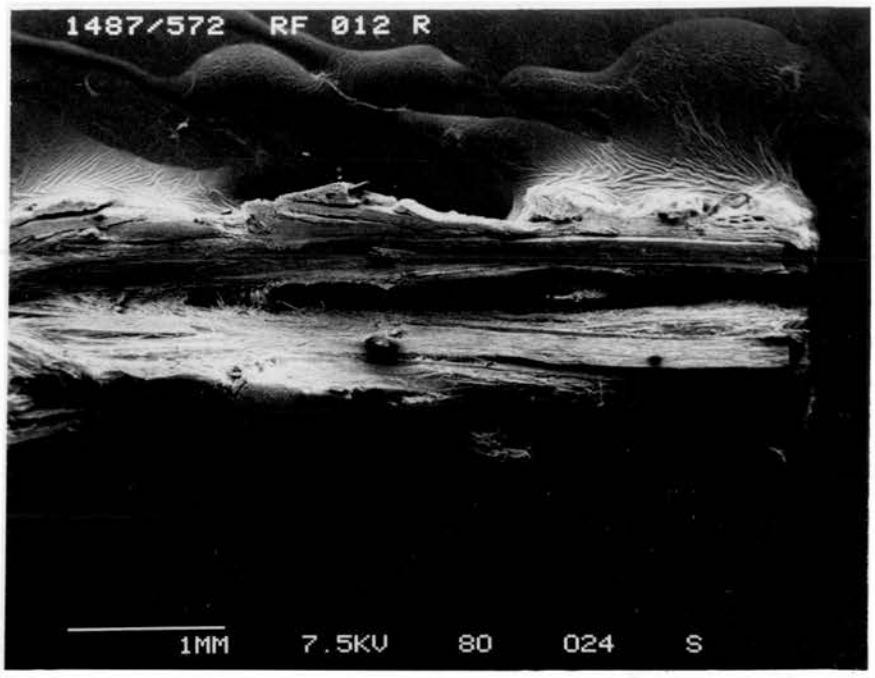

GAP VS TUBE END TO END
DISTAL FIBRE DIAMETERS
CUMULATED FREQUENCY (%)
0
20
40
60
80
100
0
6
12
18
24
30
FIBRE DIAMETER (microns)

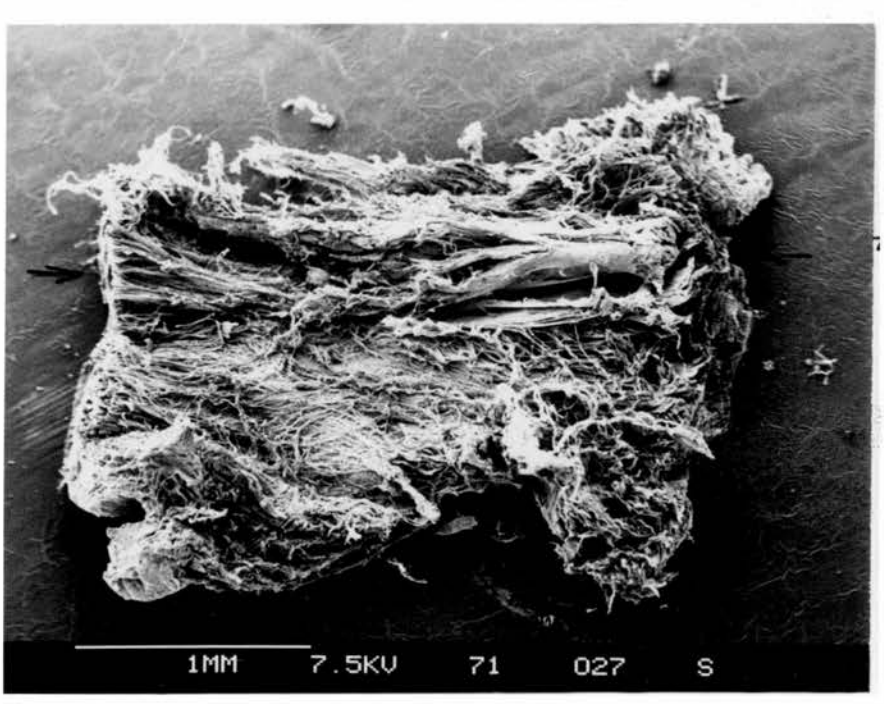
FIGURE 3.11

MEAN FIBRE DIAMETERS COMPARED
GAP vs END to END METHODS
PROXIMAL
DISTAL
MEAN FIBRE DIAMETER (microns)
0
1
2
3
4
5
6
7
8
GAP
ETHILON
VICRYL
GLUE
TUBE

FIGURE 3.12a - SCANNING ELECTRON MICROSCOPY OF THE BUCCAL BRANCH OF THE FACIAL NERVE

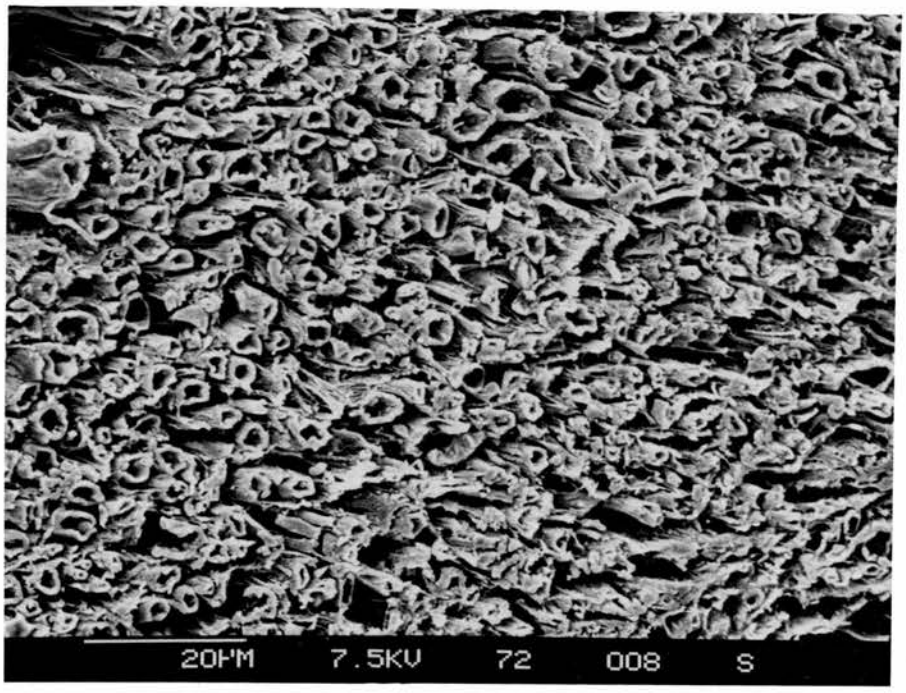


Longitudinal section - well organised axons

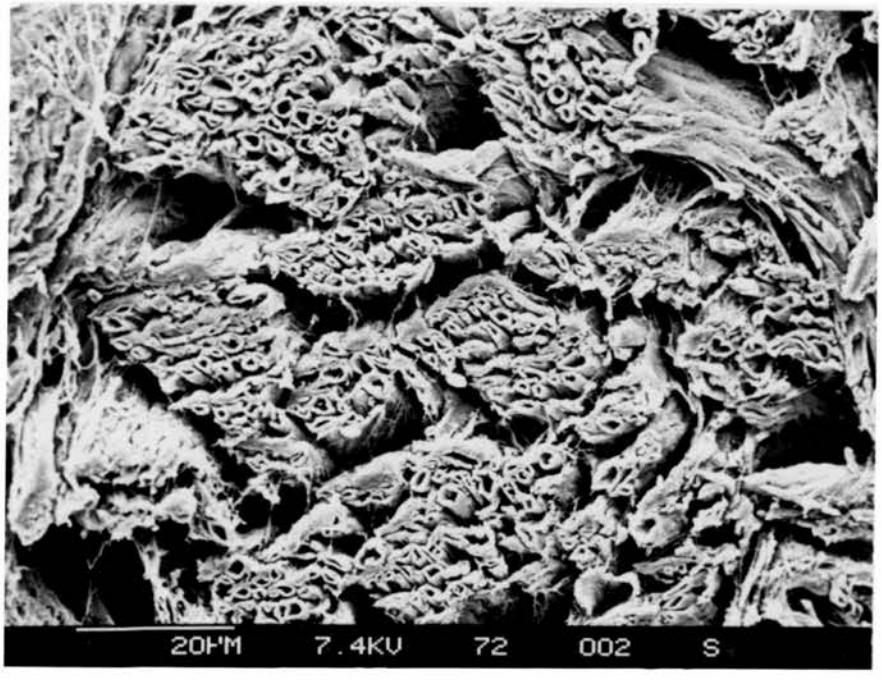


Longitudinal section - poorly organised axons

FIGURE 3.12b - SCANNING ELECTRON MICROSCOPY OF THE BUCCAL BRANCH OF THE FACIAL NERVE



Transverse section - organised axons



Transverse section - disorganised axons

anastomotic agents were laid beside the nerve to ensure there was no neurotoxic effects from the substances and a comparison between using these agents and using nothing was made. All the other methods of assessment, ie photography, electrophysiology and histology were strictly standardised. One buccal nerve was standardised with a contralateral side to avoid any interanimal variation in the size of the buccal division. The results were assessed blind and the experiment was fully randomised. The results from nerve suture in early medical literature appear contradictory and confusing. One of the factors initially overlooked was the size of the needle used for the suture. The larger the size of the needle, the greater the size of the reaction. Modern techniques have reduced the size of the needle to 70 microns, hence excite a minimal reaction. In a strict comparison of Vicryl and Ethilon, as in this experiment, only the suture materials were varied in the experiment. Ethilon is a nonabsorbable mono-filament suture of Polyamide 66 which has a high tensile strength and will not support bacterial growth or irritate tissue (Ethicon, 1985). Vicryl is an absorbable braided suture of Polyglactin 910. The suture is a copolymer of Glycolide and Lactide. The braided suture is coated with a mixture of a copolymer of Glycolide and Lactide and/or equal amounts of calcium stearate. The sutures retain 55% of their original tensile strength after 2 weeks in vivo and 20% of their tensile strength at 3 weeks. Absorption is minimal until about the 40th day postoperatively and absorption complete between the 60th and 90th day. Tisseel kit contains a human fibrin seal

which is reconstituted with aprotinin solution. Collagen film was chosen as an effective tube material as it is absorbable and non-reactive. The criteria of assessment of the results were photography, electrophysiology and histology.

Photography was used to ensure that the nerves on either side of the animal were similar in size and that all the anastomosis performed were of an acceptable standard and could be assessed not only at the time of operation but also subsequently. All the anastomosis met with the required standards. It has been reported (Sunderland, 1953) that there is a reduction in the size of the nerve as a whole after the nerve has been sectioned and particularly in the distal end. This was shown not to be the case in this experiment. The electrophysiological testing of the facial nerve in humans is contentious. No fully accepted standardised method is used worldwide. The variety of tests have been referred to in Chapter 2. The ideal electrophysiological test for the function of the facial nerve is a direct electrical stimulation with recording of the axon potential from a distal electrode. This was not feasible in this experiment as the distance between the stimulating electrode and recording electrodes is a maximum of 2 cm (the distance between where the nerve emerges from the parotid and the point at which the nerve divides into its terminal branches) and the recordings were so distorted by artifact to make understanding of the results impossible. A compromise was, therefore, sought to mimic the testing used for the human. The minimal excitibility test and

maximal excitibility tests are accepted means of testing facial nerve function. Although they do not achieve the 'gold standard', no other test has achieved this level. No significant difference was found in any of the electrophysiological testing of any of the control groups, signifying that the materials themselves do not interfere with nerve function. Electroneuronography has become popular with clinicians to assess facial nerve function. This test is fully described in Page 54 but it does not meet the full acceptance of world authorities on the subject. The test has significant sources of error, ie electrode placement variability, skin impedence, masseter muscle, artifact, patient tolerance, equipment variability, lack of standardisation, inter test variance and inter side variance (Hughes et al, 1983; Kartush et al, 1985). The most logical practical approach to facial nerve function testing in the human is to use subcutanous needles as a stimulating electrode to near the common site of the trunk of the facial nerve and stimulates appropriately. The recording electrodes are similar to those used for maximal nerve excitibility.

Histological Assessment

Preparation of the nerves for assessment has been described above and is a standard method to demonstrate the size and number of axons. When a nerve is sectioned, the axon dies off up to the nearest node of Ranvier. Wallerian degeneration occurs distally. With reinnervation across the anastomostic site there are

initially many small, immature filamentous axons which attempt to cross the site. The buccal division of the rat facial nerve is essentially a motor nerve. In this nerve, for the axon to be functional, it requires to be relatively large and the mere presence of large numbers of small immature axons apparently crossing the anastomosis, do not necessarily reflect any degree of facial nerve function. Indeed, some of these initially small axons mature and enlarge to form good mature conducting axons but the others die off or are lost with the anastomostic site (Glasby, 1989). For this reason, it is felt more appropriate not merely to count numbers of axons but to assess their size. The facial nerve is mainly motor and the larger faster nerve fibres never fully recover after neurotmesis (Craig and Thomas, 1964; Gattuso et al, 1988; Glasby et al, 1988; Glasby, 1990). The method used is described above. The present experiment was designed to standardise the use of the anastomotic material. It was not, therefore, thought relevant to histologically assess a normal buccal division of the rat facial nerve as these nerves obviously vary considerably from rat to rat. The experiment was designed to determine the comparative effects of the anastomotic materials on the nerve to determine which, if any, of the materials had a deleterious effect on the nerve. The sectioning of the nerve alone provided the control group. It is important to note that by using the buccal division, there is no tension at the anastomotic site which excludes a variable, often present in experiments involving the sciatic nerve. No difference was found between any of the groups tested using all the assessment

techniques. These findings confirm that the materials themselves do not appear to have a significant effect on the nerve. After the initial training period of handling of the materials, it was apparent there was no superiority of one anastomotic agent over the other.

3.4 CONCLUSIONS

(a) Previous experiments on the division and anastomosis of the facial nerve have failed to consider the indeterminate variables involved, ie operator variability controls and the reaction of the materials on normal nerve tissue.

(b) The absorbable suture, non-absorbable suture, glue and tube wrap used had no effect on normal nerve tissue or on the anastomosis of the sectioned facial nerve of the rat compared with simple laying together of the divided ends of the divided nerve.

TABLE 3.1 - NERVE ANASTOMOTIC AGENT AND PROCEDURE

	NERVE	ANASTOMOTIC AGENT	PROCEDURE
Rat 1.	Facial - LHS	Ethilon	Nerve cut and repaired
	- RHS	No suture	Nerve cut
Rat 2.	Facial - LHS	No suture	Nerve cut
	- RHS	Ethilon	Nerve cut and repaired
Rat 3.	Facial - LHS	Ethilon	Nerve cut and repaired
	- RHS	No suture	Nerve cut
Rat 4.	Facial - LHS	No suture	Nerve cut
	- RHS	Ethilon	Nerve cut and repaired
Rat 5.	Facial - LHS	Ethilon	Nerve cut and repaired
	- RHS	No suture	Nerve cut
Rat 6.	Facial - LHS	Vicryl	Nerve cut and repaired
	- RHS	No suture	Nerve cut
Rat 7.	Facial - LHS	No suture	Nerve cut
	- RHS	Vicryl	Nerve cut and repaired
Rat 8.	Facial - LHS	Vicryl	Nerve cut and repaired
	- RHS	No suture	Nerve cut
Rat 9.	Facial - LHS	No suture	Nerve cut
	- RHS	Vicryl	Nerve cut and repaired
Rat 10.	Facial - LHS	Vicryl	Nerve cut and repaired
	- RHS	No suture	Nerve cut

TABLE 3.1 (Continued)

	NERVE	ANASTOMOTIC AGENT	PROCEDURE
Rat 11.	Facial - LHS	Glue (Tisseel)	Nerve cut and repaired
	- RHS	No glue	Nerve cut
Rat 12.	Facial - LHS	No glue	Nerve cut
	- RHS	Glue	Nerve cut and repaired
Rat 13.	Facial - LHS	Glue	Nerve cut and repaired
	- RHS	No glue	Nerve cut
Rat 14.	Facial - LHS	No glue	Nerve cut
	- RHS	Glue	Nerve cut and repaired
Rat 15.	Facial - LHS	Glue	Nerve cut and repaired
	- RHS	No glue	Nerve cut
Rat 16.	Facial - LHS	Tube	Nerve cut and repaired
	- RHS	No tube	Nerve cut
Rat 17.	Facial - LHS	No tube	Nerve cut
	- RHS	Tube	Nerve cut and repaired
Rat 18.	Facial - LHS	Tube	Nerve cut and repaired
	- RHS	No tube	Nerve cut
Rat 19.	Facial - LHS	No tube	Nerve cut
	- RHS	Tube	Nerve cut and repaired
Rat 20.	Facial - LHS	Tube	Nerve cut and repaired
	- RHS	No tube	Nerve cut

TABLE 3.1 (Continued)

	NERVE	ANASTOMOTIC AGENT	PROCEDURE
Rat 21.	Facial - LHS	Ethilon	Nerve intact, suture
	- RHS	Vicryl	laid through the nerve
Rat 22.	Facial - LHS	Vicryl	Nerve intact, suture
	- RHS	Ethilon	laid through the nerve
Rat 23.	Facial - LHS	Ethilon	Nerve intact, suture
	- RHS	Vicryl	laid through the nerve
Rat 24.	Facial - LHS	Vicryl	Nerve intact, suture
	- RHS	Ethilon	laid through the nerve
Rat 25.	Facial - LHS	Ethilon	Nerve intact, suture
	- RHS	Vicryl	laid through the nerve
Rat 26.	Facial - LHS	Glue	No cut, anastomotic
	- RHS	Tube	agent laid next to nerve
Rat 27.	Facial - LHS	Tube	No cut, anastomotic
	- RHS	Glue	agent laid next to nerve
Rat 28.	Facial - LHS	Glue	No cut, anastomotic
	- RHS	Tube	agent laid next to nerve
Rat 29.	Facial - LHS	Tube	No cut, anastomotic
	- RHS	Glue	agent laid next to nerve
Rat 30.	Facial - LHS	Tube	No cut, anastomotic
	- RHS	Glue	agent laid next to nerve

TABLE 3.2 - A PHOTOGRAPHIC STUDY TO DETERMINE THE MACROSCOPIC EFFECT OF VARIOUS ANASTOMOTIC MATERIALS ON THE TRANSVERSE DIAMETER OF THE BUCCAL DIVISION OF THE RAT FACIAL NERVE (FASCICULAR AND EPINEURIAL REPAIR WERE ON THE RAT SCIATIC NERVE)

ANASTOMOTIC MATERIALS TESTED	WILCOXON RANK SUM TEST RESULT
Ethilon versus Vicryl	No significant difference
Glue versus no glue	No significant difference
Ethilon versus no Ethilon	No significant difference
Vicryl versus no vicryl	No significant difference
Tube versus no tube	No significant difference
Ethilon Fasc versus epi	No significant difference
Vicryl Fasc versus epi	No significant difference
Tube versus glue	No significant difference

TABLE 3.3 – AN ELECTROPHYSIOLOGICAL COMPARISON OF THE EFFECT OF VARIOUS ANASTOMOTIC AGENTS ON THE BUCCAL DIVISION OF THE RAT FACIAL NERVE (MINIMAL AND MAXIMAL EXCITABILITY TEST) [BEFORE AND AFTER ANASTOMOSIS]

Ethilon only:	Minimal	$0.5 > p > 0.1$
	Maximal	$0.5 > p > 0.1$
Vicryl only:	Minimal	$0.5 > p > 0.1$
	Maximal	$0.5 > p > 0.1$
Tube only:	Minimal	$0.5 > p > 0.1$
	Maximal	$0.5 > p > 0.1$
Glue only:	Minimal	$0.5 > p > 0.1$
	Maximal	$0.5 > p > 0.1$
Vicryl versus Cut:	Minimal	$0.5 > p > 0.1$
	Maximal	$0.5 > p > 0.1$
Ethilon versus Cut:	Minimal	$0.5 > p > 0.1$
	Maximal	$0.5 > p > 0.1$
Tube versus Cut:	Minimal	$0.5 > p > 0.1$
	Maximal	$0.5 > p > 0.1$
Glue versus Cut:	Minimal	$0.05 > p > 0.02$
	Maximal	$0.5 > p > 0.1$
Cut only:	Minimal	$0.05 > p > 0.01$
	Maximal	$0.5 > p > 0.1$

TABLE 3.4 – A STATISTICAL ANALYSIS OF THE QUALITY OF AXONS OF THE BUCCAL DIVISION OF THE RAT FACIAL NERVE TO DETERMINE THE EFFECT OF VARIOUS ANASTOMOTIC AGENTS ON THE NERVE AND A COMPARISON OF PORTIONS OF THE NERVE TO DETERMINE IF THERE IS A QUALITATIVE CHANGE ACROSS THE SITE OF THE ANASTOMOTIC AGENTS.

GROUPS		SIGNIFICANCE
Proximal Nerve	– Gap versus Ethilon	$0.5 > p > 0.1$
	– Gap versus Vicryl	$0.5 > p > 0.1$
	– Gap versus Tube	$0.5 > p > 0.1$
	– Gap versus Glue	$0.5 > p > 0.1$
Distal Nerve	– Gap versus Ethilon	$0.5 > p > 0.1$
	– Gap versus Vicryl	$0.5 > p > 0.1$
	– Gap versus Tube	$0.5 > p > 0.1$
	– Gap versus Glue	$0.5 > p > 0.1$
Proximal versus Distal Nerve	– Gap	$0.5 > p > 0.1$
	– Ethilon	$0.5 > p > 0.1$
	– Vicryl	$0.5 > p > 0.1$
	– Glue	$0.5 > p > 0.1$
	– Tube	$0.5 > p > 0.1$

TABLE 3.4 (Continued)

GROUP DESCRIPTION	T TEST RESULT
Cut versus Ethilon suture	Non significant
Cut versus no Ethilon suture	Non significant
Cut versus Vicryl suture	Non significant
Cut versus no Vicryl suture	Non significant
Cut versus tube	Non significant
Cut versus no tube	Non significant
Cut versus glue	Non significant
Cut versus no glue	Non significant
No cut and Ethilon suture	Non significant
No cut and Vicryl suture	Non significant
No cut and tube	Non significant
No cut and glue	Non significant

Students t test

Proximal Nerve	- Ethilon versus Vicryl	$0.5 > p > 0.1$
	Tube versus glue	$0.5 > p > 0.1$
Distal Nerve	- Ethilon versus Vicryl	$0.5 > p > 0.1$
	Tube versus glue	$0.5 > p > 0.1$
Proximal versus Distal	- Ethilon	$0.5 > p > 0.1$
	Vicryl	$0.5 > p > 0.1$
	Tube	$0.5 > p > 0.1$
	Glue	$0.5 > p > 0.1$

TABLE 3.5 - AN ANALYSIS OF THE QUALITY AND CROSS SECTIONAL AREA, IN SQUARE MILLIMETRES, OF AXONS IN THE BUCCAL DIVISION OF THE RAT FACIAL NERVE TO DETERMINE THE EFFECT OF VARIOUS ANASTOMOTIC AGENTS ON THE NERVE.

	GAP	ETHILON	VICRYL	GLUE	TUBE
Proximal					
Mean	6.02	5.50	5.58	5.90	6.56
SD	2.57	2.38	2.26	2.67	2.83
SEM	0.04	0.04	0.04	0.05	0.05
N	3281	3333	2755	3300	3304
Distal					
Mean	5.71	5.63	6.11	5.48	6.89
SD	2.65	2.39	2.57	2.24	3.15
SEM	0.04	0.04	0.04	0.04	0.07
N	3548	3619	3460	3338	1948

Proximal			
x		7.84	7.36
SD		3.38	3.11
SEM		0.06	0.05
N		3240	3554
Distal			
x		8.39	7.29
SD		3.83	3.19
SEM		0.07	0.06
N		3274	3367

TABLE 3.5 (Continued)

	GLUE	TUBE
x	6.26	6.39
SD	2.07	2.19
SEM	0.03	0.03
N	5937	6081

Formula Used for t test

$$t = \frac{x_1 - x_2}{\sqrt{\frac{(N_1 + N_2)}{N_1 N_2 (N_1 + N_2 - 2)} \times [N_1(SD_1)^2 + N_2(SD_2)^2]}}$$

where x_1 and x_2 = arithmetic mean

N_1 and N_2 = sample size

SD_1 and SD_2 = standard deviation

t was assessed for $(N_1 + N_2 - 2)$ degrees of freedom (Geigy, 1982)

APPENDIX 3.1

METHOD FOR PREPARATION OF RAT FACIAL NERVES FOR HISTOLOGICAL EVALUATION

Pieces of facial nerve were received from PM room already pinned out onto a marker card. The card was marked D at one side for distal, P at the other indicating proximal and a mid-line mark indicating the suture/anastomosis position. After fixation (10% buffered formalin) was complete (24 hours), the nerve was divided into 3 separate portions by cutting at a 3 mm interval either side of the suture/ anastomosis position, 3 segments of nerve were obtained. The middle segment, which contained the anastomosis, was retained for scanning electron microscopy where appropriate. The 2 remaining pieces were kept for histology.

Each piece of nerve was uniquely identified and processed through the standard Ethicol Glycol Methacrylate procedure (see over). The cut face of the nerve was orientated downwards in the methacrylate block. After the tissue was processed sections were cut at 3 um. These sections were placed onto a hot-plate to dry for a minimum of 24 hours, after which the sections were placed into a cool oven approximately 40^{o}C until it was convenient to stain them. The sections were placed in a cooler oven to prevent the sections drying out (ie, 40^{o}C). It was noted in earlier trials that placing the sections into the cool oven helped the sections to adhere to the glass slides better thereby obtaining a more uniform staining pattern.

Staining Procedure

1. Rinse sections in distilled water
2. Rinse briefly in 70% IMS
3. Transfer sections into saturated Sudan Black B in 70% alcohol for 45 minutes at room temperature.
4. Rinse briefly in 70% IMS
5. Rinse in distilled water.
6. Mount in glycerine jelly.

METHACRYLATE METHOD

Reagents

Uncatalysed Solution 'A': Take a bottle of low acid HEMA (Glycol Methacrylate). Check the quantity in millilitres using a clean measuring cylinder. For every 5 ml of low acid HEMA, add 1 ml of 2-Butoxyethanol. Mix and store in the refrigerator at 4^{o}C - this solution will keep for several months.

Catalysed Solution 'A': To 100 ml of uncatalysed Solution 'A', add Benzoil Peroxide in the quantity determined by the batch test. Place the mix in a metal beaker. Cover the beaker with cling film, place beaker in an ultra sonic bath for approximately 45 minutes, checking at 15 minute intervals. When dissolved, return the solution to the fume cupboard. This catalysed Solution 'A' must be used within one week.

Solution 'B': 15 ml Polyethylene glycol 400

1 ml NN Dimethylaniline

Mixed and stored in refrigerator

Procedure

Trim formalin fixed tissue no larger than 10 x 5 x 3 mm thick. Capsule up the fixed tissue, wrapping small specimens in Speci-wrap if necessary, ensuring that the reference number is written clearly on the capsule in pencil. Place the capsules into a large plastic beaker, cover the top of the beaker with cling or sealon film. Perforate this film with needle or forceps. Stand the beaker in a sink (ensuring there is an overflow and the plug is out), turn on the cold water tap and allow the water to fill the beaker and overflow. Leave the capsules to wash in running water for 2½ - 3 hours. After the tissue has been washed, drain off the water, dry the beaker, remove the cling/sealon film and begin the dehydration process using the following method.

Method: For small pieces of tissue no more than 3 mm square:

1½ hours in 30% industrial methylated spirit

1½ hours in 50% industrial methylated spirit

2 hours in 70% industrial methylated spirit

2 hours in 80% industrial methylated spirit

2 hours in 90% industrial methylated spirit

3 hours in 100% industrial methylated spirit

Then leave tissue in fresh 100% industrial methylated spirit overnight.

When the tissue has been dehydrated, it can be removed from the plastic capsule, placed into a clean glass vial and the vials labelled clearly with the reference number. The vials containing the tissue are to be kept covered in a dessicator from now until the end of the embedding process. The dessicator must be kept in the fume cupboard.

Commence the tissue infiltration with a 1:1 mixture of 100% industrial methylated spirit and catalysed Solution 'A', dispensing the mix into the vial using a disposable plastic Pasteur pipette, and following one of these schedules. **DO NOT LET THE TISSUE DRY OUT**

Method 'A'

For a small piece of tissue no more than 3 mm square, 3 hours in first mixture, change the mixture by pipetting the old mix from the vials and, using a clean pipette, add more mix. Repeat the process until the tissue has been infiltrated for 3 x 3 hours, leaving the tissue in the final mix overnight.

Remove the final mix using a plastic pipette. Take a clean plastic pipette and dispense pure catalysed Solution 'A' into the vials. Put the dessicator lid on, the guard into position, and put a vacuum of 400 mm Hg onto the dessicator. Leave the tissue under vacuum for

3-4 hours then release the vacuum. Repeat the process for 3 changes leaving the final change overnight.

When the infiltration with the mixes is complete, infiltration with pure catalysed Solution 'A' begins.

Remove the final mix using a plastic pipette. Take a clean plastic pipette and dispense pure catalysed Solution 'A' into the vials. Put the dessicator lid on, the guard into position, and put a vacuum of 400 mm Hg onto the dessicator. Leave the tissue under vacuum for 24 hours then release the vacuum. Repeat the process for 3 changes.

Prepare for the embedding stage in the following way:

Remove Solution 'B' from the refrigerator and allow the solution to come to room temperature. Take suitable size/sizes of clean Sorval plastic embedding trays and check that they fit inside the glass dessicator. Check that the plastic/metal stubs are clean, write the stub labels, stick onto the stubs. Take the required amount of catalysed Solution 'A' (between 2-3 ml per block dependent on size of tissue and mould size) and pipette in the required amount of Solution 'B', (the ratio determined from the batch test) using the automatic 0.5 ml pipette and disposable tip. The mixing of the 2 solutions will start the polymerisation process. Agitate the solution quickly and thoroughly using a clean plastic pipette then dispense the mixed solution into the plastic tray mould to within 1/8" of the brim. Do not leave the plastic pipette in the solution

as this may act as a secondary catalyst.

Place and orientate this tissue into the filled mould using the magnifying lamp to check the position. Lower the correctly numbered stub gently into the mould and finally pipette a small quantity of the polymerised solution down the centre hole in each stub using a clean pipette to displace any trapped air bubbles. Using a tray, carry the embedded tissue from the fume cupboard to the cabinet with the nitrogen supply. Carefully place the plastic moulds into the glass dessicator. Before replacing the dessicator lid, ensure a small air gap is maintained between the lid and the base by using a single layer of masking tape across the edge. Check that the blue plastic tube is connected to the dessicator top and to the glass tap on the jig. Turn on this glass tap checking that the second venting tap is in the off position. Ensure that there is a small quantity of sulphuric acid in the reservoir and that the hazard label is in position. Connect the blue plastic tube to the top of the reservoir. Connect to the glass rod on the jig and the second tube to the nitrogen supply line. Turn the nitrogen gas supply line on at the main stop cock to the left of the bench above floor level. Turn on the regulator situated at bench level to the left, adjusting to give just enough pressure to create gentle bubbling in the sulphuric acid reservoir. Once set up, leave running overnight.

The next morning, turn off the main nitrogen supply stop cock, release the pressure on the nitrogen regulator and shut the red supply tap to the dessicator. Gently remove the dessicator lid,

remove the trays of blocks and replace the dessicator lid. Take the tray of blocks to the fume cupboard and, wearing disposable micro touch gloves, remove the blocks from the moulds by gently rocking them to and fro. Using a small amount of IPA on a paper towel, wipe the surfaces of the methacrylate block to remove any soft or tacky methacrylate. Using a fine needle, check each block for hardness by gently pushing the needle against the methacrylate at the side of the block. At any dehydration or infiltration stage, tissue can be kept in the alcohol or infiltration mixes of catalysed Solution 'A' overnight and changed the next morning.

If the needle barely penetrates, the blocks are ready to be roughed down using a glass knife in the autocut. If the blocks are easily penetrated with the needle, place them into an oven set accurately between 58 and 60^{o}C for 1 hour only. Remove the blocks and allow to cool down. Carry out the needle check as before. Repeat this process if necessary.

APPENDIX 3.2

SAMPLE DETAILS

Sample No 9498: 0.3% collagen film, Gamma irradiated

EXPERIMENTAL PROCEDURE

2 rats per group: Survivals - 28, 49 and 70 days.

Under anaesthesia, the dorsal surface of the rat was shaved and swabbed with hibitane. A median longitudinal incision was made in the skin and access to the lumbar muscle obtained by blunt dissection. Two small pouches were made in the lumbar muscle with a Beaver blade, either side of the midline. A sample of the film (10 mm x 2.5 mm) was placed in each pouch and the pouch closed with M1.0 prolene. The skin incision was closed with interrupted stitch of M1.0 prolene. At the end of the survival period, the rats were killed with CO_2. The implants with surrounding tissue were excised and fixed in neutral buffered formalin before being processed for routine wax histology. A total of 6 SPF rats (Interfauna) were used in the study. Each rat was uniquely identified by tattoo gun.

RESULTS

Slides of the films were examined from all 3 survivals of 28, 49 and 70 days.

28 Days - In the majority of slides examined there is no definite evidence of any remnant collagen paste. Generally, the only indication of the implant sites is that of hypercellular areas within the muscle. In a couple of slides, areas of aged macrophages and collagen mark the implantation area. However, it is difficult to say if the collagen is remnant film or laid down by the host.

49 Days - Again, in the majority of slides there is no evidence of any remnant collagen film. However, in 3 of the sections there is some evidence of film, interspersed by macrophages, fibroblasts and some host collagen.

70 Days - Throughout the slides examined at this survival, there is no evidence of film, absorption being complete. In a couple of slides hupercellular areas are noted within the muscle, otherwise no indication of the implants are noted.

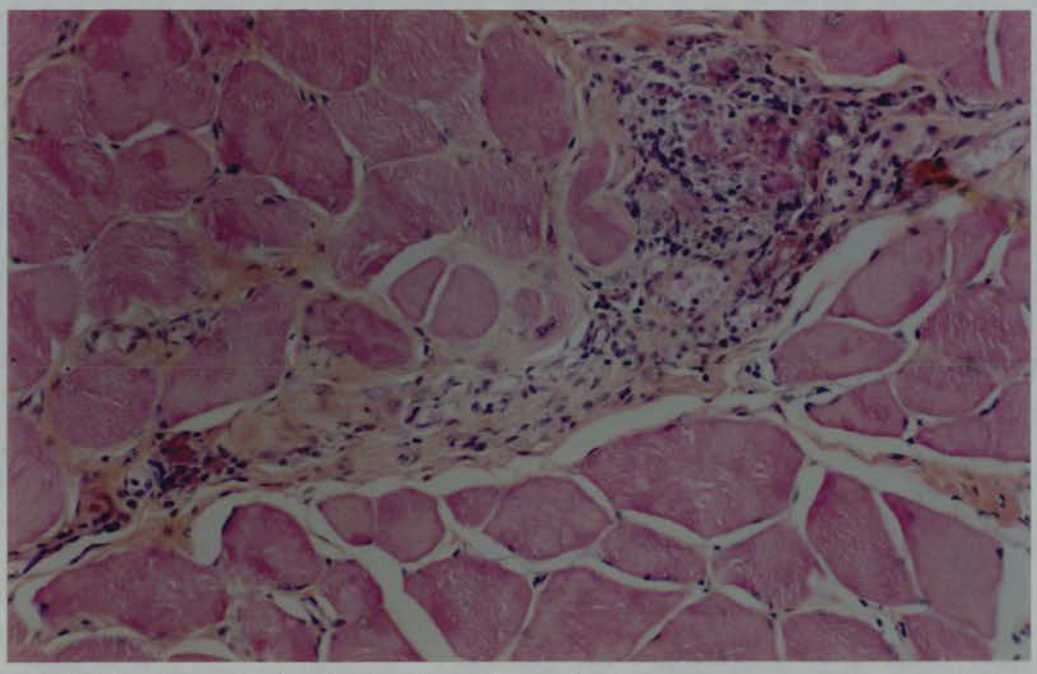
DISCUSSION AND CONCLUSIONS

It is apparent from the microscopic examination of the slides that the results are somewhat inconsistent at 28 and 49 days. There is evidence of complete absorption of the film at 28 days, however, in 3 of the 49 day sections, some of the remnant collagenous material noted does resemble collagen film. By 70 days post-implantation there is no evidence of any collagen film in the sections examined. This inconsistent behaviour of the collagen film has been noted in previous studies evaluating various preparations of collagen film.

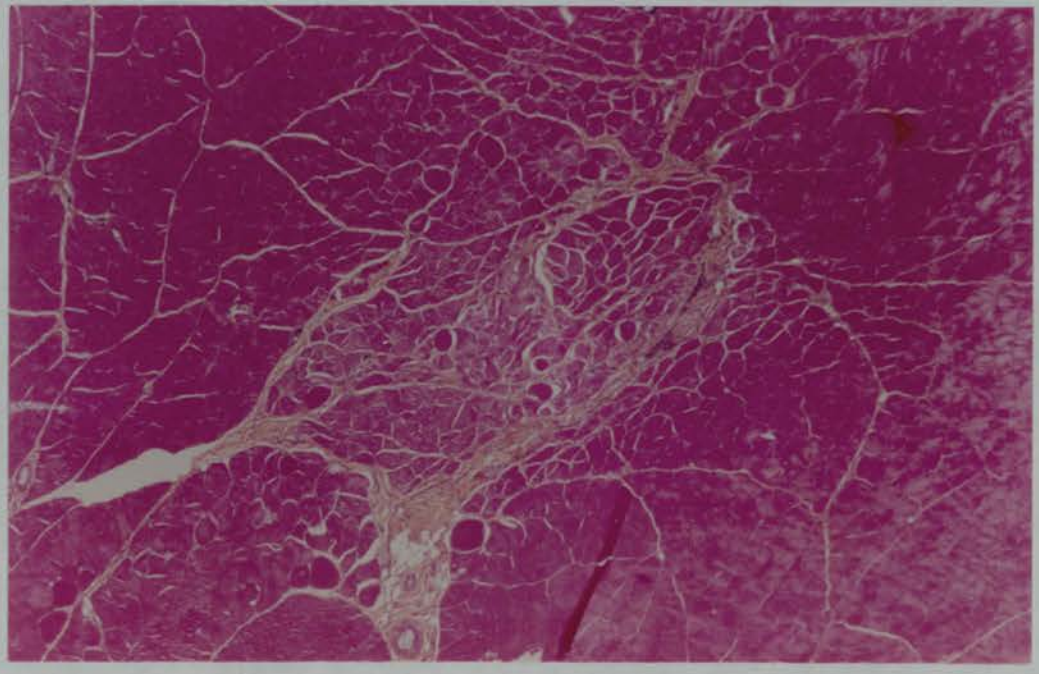
The material itself is particularly difficult to handle and implant. Taking this, and other factors into account such as individual animal variation and the small size of the study, it is only possible to make broad conclusions:-

The 0.3% collagen film tested in this study completely absorbs by 70 days post-implantation (Figure 3.10).

FIGURE 3.10 - COLLAGEN FILM, GAMMA IRRADIATED



28 days post-implantation (x 50) - area of macrophages and collagen marking the implant site



49 days post implantation (x 10) - extensive hyper-cellular area with remnant collagen film evident

APPENDIX 3.3 - EXAMPLES OF COMPUTER PRINT OUTS FOR VARIABLES OF THE EXPERIMENT (HISTOLOGICAL ANALYSIS)

EXAMPLE OF COMPUTER PRINT OUT FOR VARIABLES OF THE EXPERIMENT

(HISTOLOGICAL ANALYSIS)

Joyce-Loebl Magiscan 2 program : RESULTS Date : 1-Dec-86

Histogram of BL : [0<1]

NO TUBE VERSUS TUBE

Class	Freq	Percent
0.00000		
	0	0.00
1.20000		
	13	2.02
2.40000		
	83	12.93
3.60000		
	121	18.85
4.80000		
	94	14.64
6.00000		
	88	13.71
7.20000		
	59	9.19
8.40000		
	51	7.94
9.60000		
	38	5.92
1.08000E1		
	29	4.52
1.20000E1		
	19	2.96
1.32000E1		
	19	2.96
1.44000E1		
	17	2.65
1.56000E1		
	4	0.62
1.68000E1		
	3	0.47
1.80000E1		
	0	0.00
1.92000E1		
	2	0.31
2.04000E1		
	1	0.16
2.16000E1		
	0	0.00
2.28000E1		
	0	0.00
2.40000E1		
	1	0.16
2.52000E1		
	0	0.00
2.64000E1		
	0	0.00
2.76000E1		
	0	0.00
2.88000E1		
	0	0.00
3.00000E1		

RG 294L PG085 FN A

Sample identifier : RG 294L PG085 FN A
Reference number : 0

File name : MURRAY217
File date : 1-Oct-86

Data file author : GILMOUR
Comment : MICRONS

EXAMPLE OF COMPUTER PRINT OUT FOR VARIABLES OF THE EXPERIMENT

(HISTOLOGICAL ANALYSIS)

-STATISTICS SUMMARY

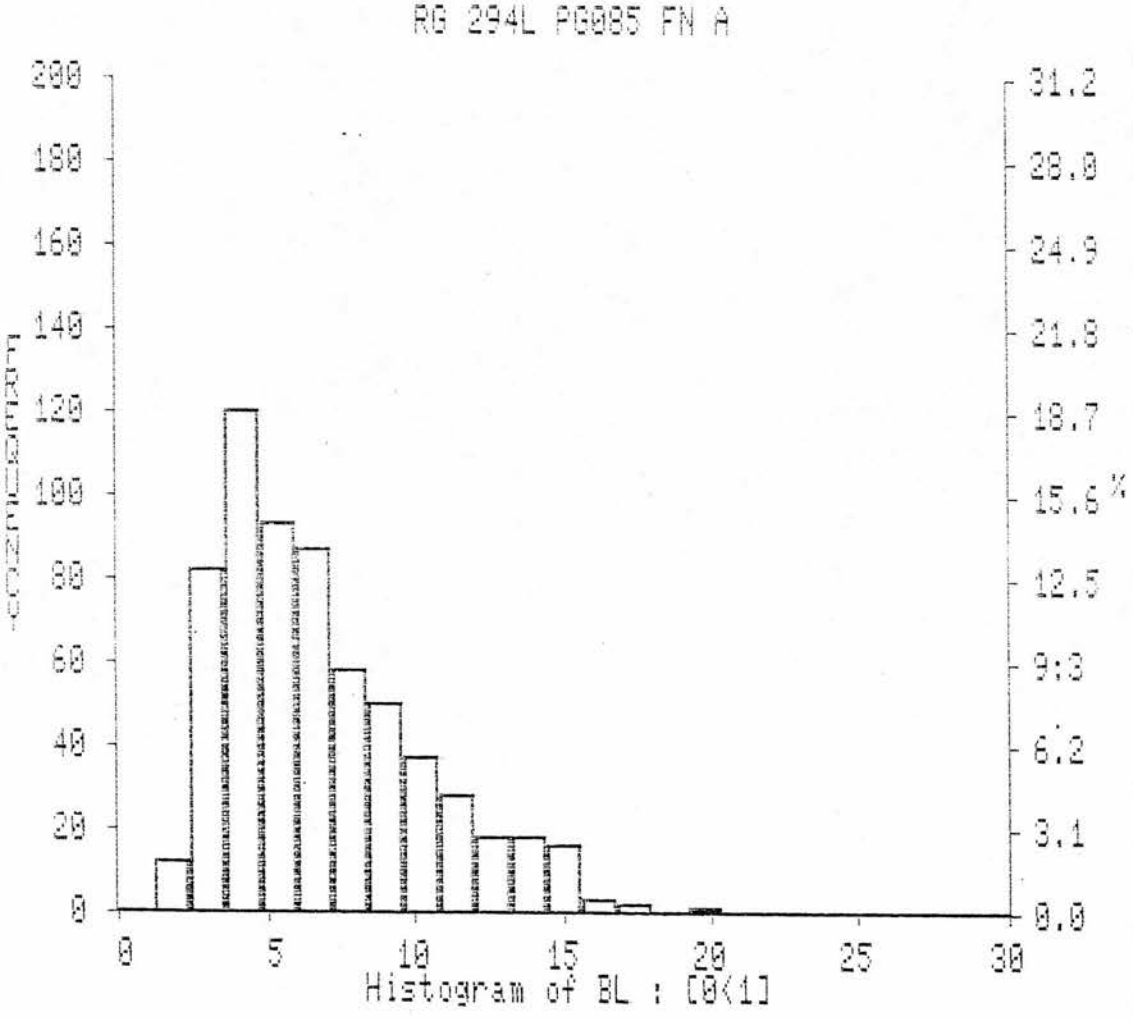
NO TUBE VERSUS TUBE

	MIN	MAX	TOTAL	MEAN	STD. DEV
AREA:	5.85399	1.21955E2	1.39640E4	2.17507E1	1.81997E1
LENG:	1.69015	2.41992E1	4.48400E3	6.98442	3.54007
BRED:	2.09314	1.51187E1	3.22824E3	5.02841	2.32993
PERI:	6.23480	7.13441E1	1.27752E4	1.98990E1	1.28944E1
NOBJ:	3.86000E2	3.36000E2	6.42000E2	3.21000E2	1.50000E1
TARE:	2.37489E4	2.41660E4	4.79149E4	2.39575E4	2.08614E2
%TARE:	4.25594E1	4.33058E1	8.58643E1	4.29321E1	

Frame area = 5.58031E4

From 642 object(s) in 2 field(s).
Units: MICRONS Scale: 4.68763E-1 / pixel

Joyce-Loebl Magiscan 2 program : RESULTS Date : 1-Dec-86



Sample identifier : RG 294L PG085 FN A
Reference number : 0

File name : MURRAY217
File date : 1-Oct-86

Data file author : GILMOUR
Comment : MICRONS

EXAMPLE OF COMPUTER PRINT OUT FOR VARIABLES OF THE EXPERIMENT

(HISTOLOGICAL ANALYSIS)

Joyce-Loebl Magiscan 2 program : RESULTS Date : 1-Dec-86

STATISTICS

*** STATISTICS OF BL : [0<1] ***

over 642 objects.

Sample identifier: RG 294L P6085 FN A

Minimum =	1.69015
Maximum =	2.41992E1
Total value =	4.48400E3
Mean =	6.98442
Geometric mean =	6.19182
Harmonic mean =	5.49446
Sample variance =	1.25321E1
Sample standard deviation =	3.54007
Population variance =	1.25516E1
Population standard deviation =	3.54283
Standard deviation of the mean =	1.39715E-1
Relative standard error =	2.00039E-2
Skew =	5.00262E1
Kurtosis =	6.66936E2

Sample identifier : RG 294L P6085 FN A
Data file author : GILMOUR
Reference number : 0

File name : MURRAY217
File date : 1-Oct-86
Comment : MICRONS

NO TUBE VERSUS TUBE

4. ABSORBABLE AND NONABSORBABLE SUTURES

4.1 INTRODUCTION

The suture material used to anastomose the divided peripheral nerve remains debatable. In principle it is thought that non-absorbable material is preferable to absorbable material because it excites a lesser reaction. Improved technology has reduced the size of the needle to 70 microns (Ethicon) and the normal suture material size is now 10/0. Many of the previously reported results used larger sutures and needles and the materials used were not so refined and non-antigenic as are modern sutures. This experiment compares the use of an absorbable suture material (Vicryl - Ethicon) and a non-absorbable suture (Ethilon - Ethicon) to anastomose the divided buccal division of the rat facial nerve.

4.2 METHODS AND MATERIALS

The general method of preparation, operative technique and assessment of results has been indicated in Chapter 3 and this experiment followed closely the outline indicated as such. The buccal nerve was divided transversely and immediately the 2 ends anastomosed with epineurial sutures placed at 6 and 12 o'clock. The sutures used were randomised, one side had the absorbable suture and the other side the non-absorbable suture. The nerve was photographed before and after the procedure and at 10 weeks post-operatively the electrophysiological assessment was identical as that described in Chapter 3 and the histological assessment was also conducted along similar lines. In addition, the actual anastomotic site was taken and viewed under the scanning electron microscope. The pictures obtained from the quality of axons proximal and distal to the anastomosis were assessed.

4.3 RESULTS

Twenty live Sprague-Dawley rats were used. They were 7 to 8 weeks old and weighed between 200 and 300 gm.

Photography

It was confirmed by photography that the preoperative size of the buccal nerve was similar on both sides of the animal. The quality of the anastomosis was assessed by 2 observers immediately after the suture and no significant difference was found between the materials (Wilcoxon Rank sum test) (Tables 3.2, 4.1 and 4.2). At post-mortem the nerves were again photographed. There was no significant loss in overall diameter of the nerves and there was no significant difference in the overall size of the nerves comparing the absorbable and non-absorbable suture. The anastomotic site was assessed qualitatively and no significant difference was found between the 2 materials (Tables 3.2, 4.1 and 4.2).

A correlation between observer quality of anastomosis and axon count difference across the anatomosis (Table 4.2) showed there was no significant superiority of Vicryl over Ethilon.

Electrophysiology

The minimal excitability test showed no significant difference between the 2 materials. Similarly the maximal excitability test showed no difference between the 2 materials (Tables 4.3 and 4.4).

Histological Assessment

For the experiment above, both the axon counts and the semi-qualitative assessment referred to in Chapter 3 were studied (Fig 4.1). The axon counts were studied as a percentage change across the anastomosis. The log of the axon count proximally and the log of the axon count distally was taken and the difference identified as a relative ratio between the two. There is no significant difference between Ethilon and Vicryl (Table 4.5). The cross sectional area of the axons within the cross sectional area of the total nerve were studied and the percentage difference noted. Using the Wilcoxon Rank sum test, the correlation of the area is depending on the suture was computed but there was no significant difference between the 2 (Table 4.6). The density of the axons and axons per square micron within the perineurial sheath was also noted in both the proximal and distal sections to the anastomosis. The percentage difference was then taken and the Wilcoxon Rank sum test showed there was no significant difference between the 2 sutures (Tables 4.7 and 4.8). A correlation between observer quality of

FIGURE 4.1 - TRANSVERSE SECTION OF BUCCAL DIVISION OF THE RAT FACIAL NERVE FOR LIGHT MICROSCOPY

anastomosis and axon count difference across the anastomosis showed no significant difference.

The semi-qualitative assessment was undertaken using the Magiscan as detailed in Chapter 3. This showed no significant difference between the 2 materials (Student's t test) (Table 4.9, Figs 4.2 and 4.3).

Scanning Electron Microscopy

A qualitative assessment of the state of the axons failed to show any significant difference between the 2 materials (Wilcoxon Rank sum test) (Fig 4.4 and Appendix 4.1). The photographs of the scanning electron microscopy of the longitudinal and transverse sections of the nerves were studied. They were placed in order of neatness from best to worst. The actual suture used was unknown to the observer. The photographs were then ranked. The Wilcoxon Rank sum test was then applied and there was no significant difference between the 2 materials used. A quantitative assessment of the axons was not possible.

FIGURE 4.2

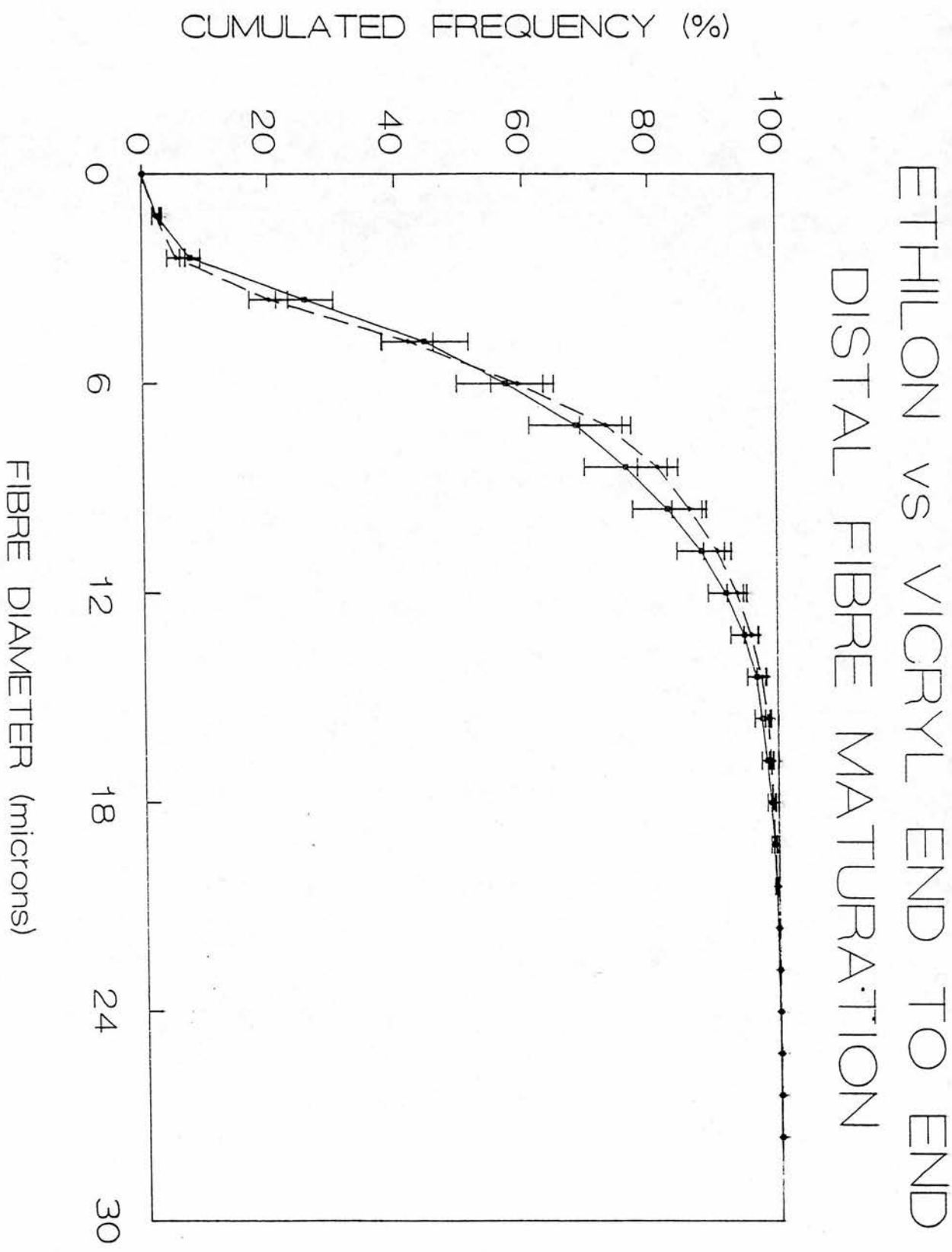
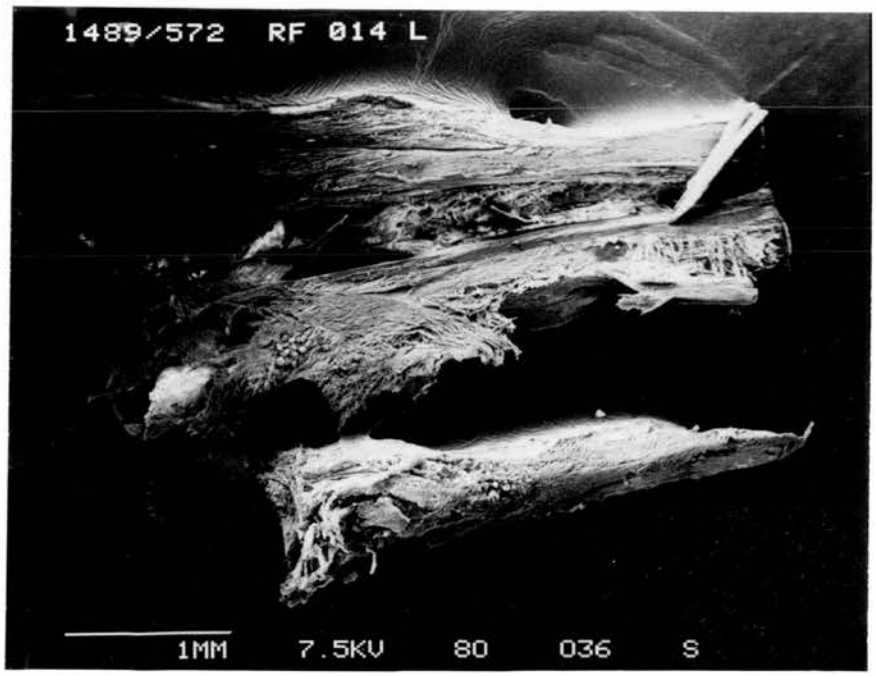

ETHILON VS VICRYL END TO END
DISTAL FIBRE MATURATION
CUMULATED FREQUENCY (%)
0
20
40
60
80
100
0
6
12
18
24
30
FIBRE DIAMETER (microns)

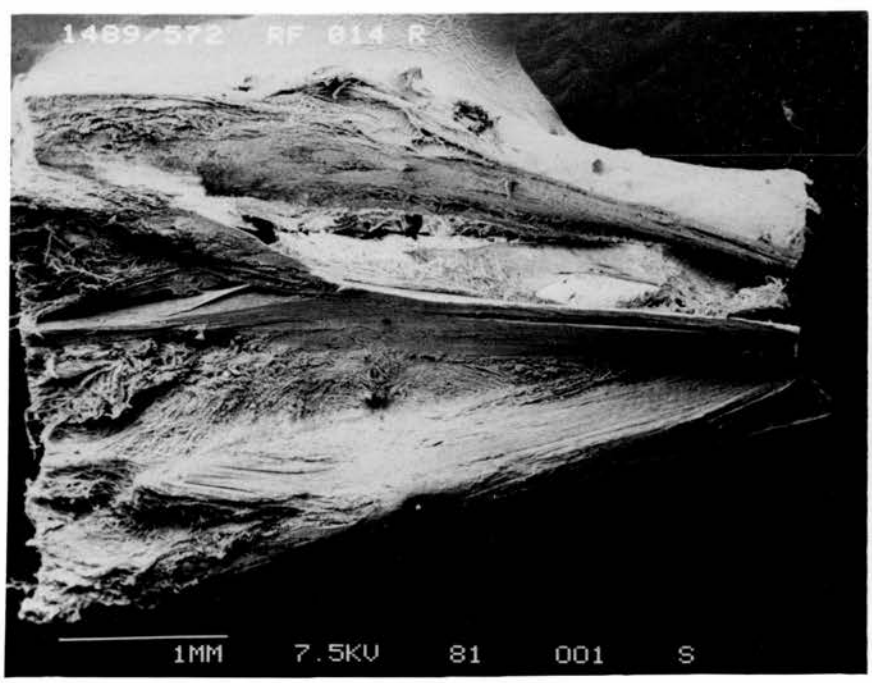
FIGURE 4.3

ETHILON VS VICRYL END TO END
PROXIMAL & DISTAL FIBRES COMPARED
PROXIMAL
DISTAL
MEAN FIBRE DIAMETER (microns)
0
1
2
3
4
5
6
7
ETHILON
VICRYL

FIGURE 4:4 - SCANNING ELECTRON MICROSCOPY OF THE BUCCAL DIVISION OF THE RAT FACIAL NERVE



Longitudinal section - Absorbable sutures



Longitudinal section - Non absorbable sutures

4.4 DISCUSSION

The use of absorbable sutures for peripheral nerve repair has, in general, been avoided. The origins of this aversion are found in medical literature relating to nerve repair, particularly after the experiences of surgeons during the 2 World Wars (see Chapter 2). The main argument against absorbable sutures as opposed to non-absorbable sutures is the size of the delayed reaction which is considerably greater for absorbable sutures (Sunderland, 1978).

Most of these studies, however, use materials which are reactive and antigenic. From Chapter 3, the results of the control studies indicate that the non-absorbable suture used in this experiment has minimal, if any, reactive qualities 10 weeks after the suture has been placed. There should be no theoretical contraindication to the use of this non-absorbable suture for nerve repair. The results of this experiment confirms this. In contrast, Lee et al (1983) reported that the reanastomosis of the freshly severed rat sciatic nerve, using Dexon and nylon 9/0 interrupted epineurial sutures did not produce an identical histological result. There were almost identical suture reactions by day 7 except that the intensity of the polymorphonuclear and macrophage infiltrate in the nylon sutured nerves was far greater than in the Dexon sutured group. Although the intensity of the nylon suture reaction subsided considerably after day 21, the lymphocytes, macrophages and

plasma cells surround the suture as long as the sutures remain in the nerves up to 12 months postoperatively. The Dexon sutured nerve segment showed disappearance of inflammatory cells as soon as the sutures were completely absorbed. The Dexon sutures were still detectable up to 2 months but not after 3 months. This group studied the histological reaction to the suture after anastomosis whereas in the experiment described in this chapter a much better functional assessment was performed.

4.5 CONCLUSIONS

(a) There does not appear to be any significant difference in the outcome of anastomosing the divided buccal division of the rat facial nerve with Ethilon 10/0 suture (non-absorbable) and Vicryl 10/0 suture (absorbable) as assessed by photographic, histological or electrophysiological means.

TABLE 4:1 - A COMPARISON OF THE TRANSVERSE DIAMETER OF THE NERVE PROXIMAL AND DISTAL TO THE ANASTOMOSIS RELATIVE TO THE TRANSVERSE DIAMETER PREOPERATIVELY

	PREOPERATIVE			POSTOPERATIVE		RESULT		ETHILON		SIGNED
AT	P TD	D TD	DIFF	P DIA	D DIA	DIFF	SUTURE	-VICRYL	RANK	RANK
1L	70 mm	70 mm	0	70 mm	60 mm	10	E	-20	13	-13
1R	50 mm	50 mm	0	80 mm	60 mm	20	V			
2L	80 mm	70 mm	10	115 mm	80 mm	25	V	+20	13	+13
2R	60 mm	50 mm	10	100 mm	95 mm	-5	E			
3L	110 mm	90 mm	20	140 mm	110 mm	10	V	+5	2½	+2½
3R	105 mm	90 mm	15	190 mm	180 mm	-5	E			
4L	80 mm	70 mm	10	110 mm	45 mm	55	E	55	17	+17
4R	65 mm	50 mm	15	80 mm	65 mm	0	V			
5L	70 mm	70 mm	0	90 mm	60 mm	30	E	5	2½	+2½
5R	65 mm	55 mm	10	95 mm	60 mm	25	V			
6L	80 mm	70 mm	10	110 mm	95 mm	5	V	15	8	+8
6R	60 mm	60 mm	0	70 mm	50 mm	20	E			
7L	60 mm	50 mm	10	95 mm	80 mm	5	E	5	2½	+2½
7R	65 mm	60 mm	5	95 mm	90 mm	0	V			
8L	70 mm	70 mm	0	100 mm	100 mm	0	V	30	16½	+16½
8R	50 mm	50 mm	0	100 mm	70 mm	30	E			
9L	100 mm	70 mm	30	105 mm	95 mm	-10	V	-10	5	-5
9R	80 mm	60 mm	20	110 mm	110 mm	-20	E			
0L	60 mm	50 mm	10	85 mm	75 mm	0	E	0		
0R	50 mm	50 mm	0	90 mm	90 mm	0	V			
1L	40 mm	40 mm	0	110 mm	80 mm	30	E	20	13	+13
1R	40 mm	40 mm	0	95 mm	85 mm	10	V			
2L	40 mm	40 mm	0	65 mm	60 mm	5	V	15	8	+8
2R	50 mm	50 mm	0	80 mm	60 mm	20	E			
3L	40 mm	40 mm	0	60 mm	60 mm	0	V	15	8	+8
3R	40 mm	40 mm	0	110 mm	95 mm	15	E			

'ABLE 4:1 (continued)

	PREOPERATIVE			POSTOPERATIVE		RESULT		ETHILON		SIGNED
AT	P TD	D TD	DIFF	P DIA	D DIA	DIFF	SUTURE	-VICRYL	RANK	RANK
4L	50 mm	50 mm	0	110 mm	70 mm	40	V	-20	13½	-13
4R	80 mm	80 mm	0	110 mm	90 mm	20	E			
5L	50 mm	45 mm	5	125 mm	100 mm	20	E	15	8	+8
5R	40 mm	40 mm	0	50 mm	45 mm	5	V			
6L	55 mm	50 mm	5	110 mm	80 mm	25	E	15	8	+8
6R	50 mm	50 mm	0	100 mm	90 mm	10	V			
7L	50 mm	50 mm	0	115 mm	95 mm	20	V	-20	13½	-13
7R	55 mm	50 mm	5	110 mm	105 mm	0	E			
9L	60 mm	60 mm	0	50 mm	50 mm	0	V	30	16½	+16½
9R	60 mm	60 mm	0	100 mm	70 mm	30	E			
0L	90 mm	90 mm	0	100 mm	85 mm	15	E	-5	2½	-2½
0R	80 mm	80 mm	0	100 mm	80 mm	20	V			

at 18 unsuitable for assessment

rojected onto screen from 20 metres - size of slide 23 x 35 mm
size on screen 1200 x 1800 mm

(-) = 46.5 for 18 pairs, ie NS

(+) = 123.5

ilcoxon Signed Rank Sum Test is non-significant

= proximal

= distal

D = transverse diameter

TABLE 4.2a - OBSERVER VS DIFFERENCE IN AXON COUNT (n = 17)

Correlation of Vicryl

NO	OBSERVERS (X)	LOG (Y)	X^2	Y^2	X x Y
1	39	0.197	152	0.039	7.68
2	45	0.134	2025	0.018	6.03
3	40	0.117	1600	0.014	4.68
4	4	0.191	16	0.036	0.764
5	21	0.035	441	0.0012	0.735
6	4	0.385	16	0.148	1.54
7	0	0.176	0	0.031	0
8	48	0.196	2304	0.038	9.41
9	60	0.586	3600	0.343	35.16
10	48	0.008	2304	0.00	0.38
11	42	0.04	1764	0.002	1.68
12	90	0.24	8100	0.58	21.6
13	90	0.356	8100	0.127	32.04
14	56	0.809	3136	0.654	45.3
15	30	0.065	900	0.004	1.95
16	48	0.042	2304	0.002	2.02
19	100	0.168	10000	0.028	16.8
	765	3.745	46762	2.065	187.77

Rat 17, 18 and 20 unsuitable for assessment

Wilcoxon Signed Rank Sum Test is non-significant

TABLE 4.2b - OBSERVER VS DIFFERENCE IN AXON COUNT (n= 17)

Correlation of Ethilon

NO	OBSERVERS (X)	LOG (Y)	X^2	Y^2	X x Y
1	27	0.192	729	0.0369	5.184
2	60	1.004	3600	1.008	60.24
3	30	0.330	900	0.1089	9.9
4	7	0.631	49	0.398	4.417
5	60	0.167	3600	0.0279	10.02
6	5	0.049	25	0.002	0.245
7	60	0.028	3600	0.0001	1.68
8	40	0.278	1600	0.077	11.12
9	60	0.340	3600	0.116	20.4
10	33	0.204	1089	0.042	6.732
11	30	0.263	900	0.069	7.89
12	18	0.352	324	0.124	6.34
13	0	0.314	0	0.099	0
14	39	0.269	1521	0.0724	10.49
15	16	1.225	256	1.50	19.6
16	14	1.038	196	1.077	14.53
19	76	0.343	5776	0.1187	26.07
	614	7.027	27765	4.876	214.86
Mean	36.12	0.413			

Rat 17, 18 and 20 unsuitable for assessment

Wilcoxon Signed Rank Sum Test is non-significant

TABLE 4.2c - A CORRELATION OF OBSERVER VS DIFFERENCE IN AXON ACROSS THE ANASTOMOSIS.
VICRYL AND ETHILON SEPARATELY ASSESSED

RAT	OBSERVER FIGURE PHOTO	LOG OF PROXIMAL AND DISTAL AXON COUNT	SUTURE
1L	27	0.192	E
1R	39	0.197	V
2L	45	0.134	V
2R	60	1.004	E
3L	40	0.117	V
3R	30	0.330	E
4L	7	0.631	E
4R	4	0.191	V
5L	60	0.167	E
5R	21	0.035	V
6L	4	0.385	V
6R	5	0.049	E
7L	60	0.028	E
7R	0	0.176	V
8L	48	0.196	V
8R	40	0.278	E
9L	60	0.586	V
9R	60	0.340	E
10L	33	0.204	E
10R	48	0.008	V

TABLE 4.2c (continued)

RAT	OBSERVER FIGURE PHOTO	LOG OF PROXIMAL AND DISTAL AXON COUNT	SUTURE
11L	30	0.263	E
11R	42	0.040	V
12L	90	0.240	V
12R	18	0.352	E
13L	90	0.356	V
13R	0	0.314	E
14L	56	0.809	V
14R	39	0.269	E
15L	16	1.225	E
15R	30	0.065	V
16L	14	1.038	E
16R	48	0.042	V
17L	42	0.174	V
17R	14	0.096	E
18L	20		
18R	--		
19L	100	0.168	V
19R	76	0.343	E
20L	72		
20R	--		

Rat 18 and 20 unsuitable for assessment

Wilcoxon Signed Rank Sum Test is non-significant

TABLE 4.2d - CORRELATION RANK SUM TEST TO COMPARE EFFICACY OF VICRYL AND ETHILON IN THE NEATNESS OF THE ANASTOMOSIS AND AXON COUNT ACROSS THE ANASTOMOSIS

Vicryl

$$t = r \sqrt{\frac{n-2}{1-r^2}}$$

$$= 0.156 \sqrt{\frac{15}{0.976}}$$

$$= 0.61 \quad (p > 0.5) \qquad \text{ie, non-signficant}$$

Ethilon

to test deviation r from 0 on nil correlation

$$t = r \sqrt{\frac{n-2}{1-r^2}} = 0.88$$

$$0.5 > p > 0.1 \qquad \text{ie, non-significant}$$

TABLE 4.3 - MINIMAL EXCITABILITY TEST: A comparison of sutures with each other. Each nerve stimulated before division + at 10 weeks and the difference taken in volts.

RAT	ETHICON DIFFERENCES	VICRYL DIFFERENCES	ETHILON-VICRYL	RANK	SIGNED RANK
1	-1	6	-7	13	-13
2	37	19	18	16	+16
3	2	6	-4	9	-9
4	8	3	5	11½	+11½
5	5	5	0		
6	7	7	0		
7	-1	-1	0		
8	0	1	-1	2½	-2½
9	-1	-2	+1	2½	+2½
10	4	3	1	2½	+2½
11	1	9	-8	14	-14
12	4	1	3	6½	+6½
13	3	0	3	6½	+6½
14	-1	1	-2	5	-5
15	-3	2	-5	11½	-11½
16	7	3	4	9	+9
17	0	-1	+1	2½	+2½
19	1	10	-9	15	-15
20	10	6	4	9	+9

Rat 18 unsuitable for assessment T(-) 70
T(+) 60

Wilcoxon Signed Rank Sum Test is non-significant

TABLE 4.4 - MAXIMAL EXCITABILITY TEST: A comparison of Ethicon and Vicryl. Each nerve is stimulated before division and at 10 weeks postop and the difference taken in volts.

RAT	ETHICON DIFFERENCES	VICRYL DIFFERENCES	ETHILON-VICRYL	RANK	SIGNED RANK
1	0	-12	+12	13	+13
2	44	37	7	6½	+6½
3	-3	-17	+14	15	+15
4	-17	9	-26	17	-17
5	0	7	-7	6½	-6½
6	11	0	11	12	+12
7	-11	-6	-5	3½	-3½
8	-8	2	-10	10½	-10½
9	+2	15	-13	14	-14
10	18	10	8	9	+9
11	-3	29	-32	18	-18
12	-23	-8	-15	16	-16
13	-3	2	-5	3½	-3½
14	8	15	-7	6½	-6½
15	-22	-12	-10	10½	-10½
16	7	14	-7	6½	-6½
17	-7	-11	+4	2	+2
19	14	-19	33	19	+19
20	10	11	-1	1	-1

Rat 18 unsuitable for assessment T(-) = 113.5
T(+) = 76.5

Wilcoxon Signed Rank Sum Test is non-significant

TABLE 4.5 - AXON COUNTS: Percentage change across anastomosis expressed as a log and a strict comparison of Ethilon versus Vicryl.

RAT	PROXIMAL	DISTAL	LOG P	LOG D	DIFF	SUTURE	ETHILON -VICRYL	RANK	SIGNED RANK
1L	1712	2667	3.234	3.426	0.192	E	-0.005	1	-1
1R	2080	3277	3.318	3.515	0.197	V			
2L	2536	3453	3.404	3.538	0.134	V	0.87	16	+16
2R	2189	217	3.340	2.336	1.004	E			
3L	3097	2367	3.491	3.374	0.117	V	0.447	14	+14
3R	1688	3603	3.227	3.557	0.330	E			
4L	574	2453	2.759	3.390	0.631	E	0.440	13	+13
4R	4853	3129	3.686	3.495	0.191	V			
5L	2688	3941	3.429	3.556	0.167	E	0.132	5	+5
5R	2468	2671	3.392	3.427	0.035	V			
6L	3338	1375	3.523	3.138	0.385	V	-0.336	12	-12
6R	2588	2312	3.413	3.364	0.049	E			
7L	1901	2026	3.279	3.307	0.028	E	-0.148	6½	-6½
7R	3134	2088	3.496	3.320	0.176	V			
8L	2451	3850	3.389	3.585	0.196	V	0.082	3	+3
8R	3505	1851	3.545	3.267	0.278	E			
9L	1407	5426	3.148	3.734	0.586	V	-0.246	11	-11
9R	1843	4032	3.266	3.606	0.340	E			
10L	2847	4555	3.454	3.658	0.204	E	0.196	9	+9
10R	2527	2574	3.403	3.411	0.008	V			

P = proximal

D = distal

TABLE 4:5 (continued)

RAT	PROXIMAL	DISTAL	LOG P	LOG D	DIFF	SUTURE	ETHILON -VICRYL	RANK	SIGNED RANK
11L	1425	2615	3.154	3.417	0.263	E	0.223	10	+10
11R	2998	2735	3.477	3.437	0.040	V			
12L	1532	2658	3.185	3.425	0.240	V	0.112	4	+4
12R	1528	3432	3.184	3.536	0.352	E			
13L	3072	1351	3.487	3.131	0.356	V	0.042	2	+2
13R	1190	2456	3.076	3.390	0.314	E			
14L	2107	327	3.324	2.515	0.809	V	-0.54	15	-15
14R	1919	3562	3.283	3.552	0.269	E			
15L	257	4312	2.410	3.635	1.225	E	1.16	18	+18
15R	1908	1645	3.281	3.216	0.065	V			
16L	252	2748	2.401	3.439	1.038	E	0.996	17	+17
16R	1940	2137	3.288	3.330	0.042	V			
19L	3680	2498	3.566	3.398	0.168	V	0.175	8	+8
19R	1964	4329	3.293	3.636	0.343	E			
20L	1998	3325	3.301	3.522	0.221	E	0.148	6½	+6½
20R	2140	1807	3.330	3.257	0.073	V			

Rat 17 and 18 unsuitable for assessment.

The nearest root the best, therefore, the difference is always + ve

$T(-) = 45.5 = NS$

Wilcoxon Signed Rank Sum Test is non-significant $T(+) = 125.5 = NS$

TABLE 4.6 - AREA OF AXONS: Percentage area of axons of total nerve proximal and distal.

NERVE	PERCENTAGE AREA PROXIMAL	PERCENTAGE AREA DISTAL	PERCENTAGE DIFFERENCE	SUTURE	ETHILON -VICRYL	RANK	SIGNED RANK
1L	26.29	18.00	8.29	E	-11.21	12	-12
1R	29.80	10.30	19.50	V			
2L	13.23	17.21	3.98	V	+5.42	9	+9
2R	16.14	6.74	9.40	E			
3L	52.57	16.30	36.27	V	-29.83	17	-17
3R	13.58	7.14	6.44	E			
4L	6.4	28.1	21.7	E	16.38	14	+14
4R	12.4	17.72	5.32	V			
5L	7.00	17.62	10.62	E	4.9	8	+8
5R	29.03	23.31	5.72	V			
6L	25.89	15.16	10.73	V	22.01	16	+16
6R	38.66	5.92	32.74	E			
7L	12.95	11.63	1.32	E	-15.1	13	-13
7R	25.16	8.74	16.42	V			
8L	8.37	7.58	0.79	V	0.28	1	+1
8R	10.68	11.75	1.07	E			
9L	13.10	6.5	6.6	V	1.2	3	+3
9R	4.27	12.07	7.80	E			
10L	12.72	6.85	5.37	E	-4.57	7	-7
10R	19.31	9.37	9.94	V			

TABLE 4.6 - Continued

NERVE	PERCENTAGE AREA PROXIMAL	PERCENTAGE AREA DISTAL	PERCENTAGE DIFFERENCE	SUTURE	ETHILON -VICRYL	RANK	SIGNED RANK
11L	14.33	8.62	5.71	E	-38.03	18	-18
11R	51.67	7.93	43.74	V			
12L	9.83	9.35	0.50	V	21.05	15	+15
12R	45.93	24.38	21.55	E			
13L	10.85	10.69	0.18	V	0.78	2	+2
13R	8.80	7.84	0.96	E			
14L	8.27	1.70	6.57	V	11	11	+11
14R	25.57	8.00	17.57	E			
15L	10.44	18.98	8.54	E	6.98	10	+10
15R	18.59	20.15	1.56	V			
16L	14.56	19.35	4.79	E	3.79	6	+6
16R	11.56	12.56	1.00	V			
19L	21.18	12.68	8.50	V	-3.27	5	-5
19R	12.07	6.84	5.23	E			
20L	10.85	6.54	4.31	E	-2.82	4	-4
20R	4.30	11.43	7.13	V			

Rat 17 and 18 unsuitable for assessment

Wilcoxon Signed Rank Sum Test is non-significant T(-) = 76 = NS
T(+) = 95 = NS

TABLE 4.7 - AXON DENSITY IN AXONS PER SQUARE MICRON IN PERINEURIUM

	PROXIMAL			DISTAL			
NERVE	AXONS	AREA	DENSITY	AXONS	AREA	DENSITY	PERCENTAGE DIFFERENCE
1L	1712	95327	0.01796	2667	109419	0.02437	35.70 E
1R	2080	212251	0.00980	3277	114129	0.02871	192.96 V
2L	2536	134276	0.01890	3435	77274	0.04445	135.19 V
2R	2189	89291	0.02452	217	52760	0.00411	496.60 E
3L	3097	10236	0.30256	2367	161987	0.01461	1970.91 V
3R	1688	11520	0.14653	3603	90695	0.03973	269.96 E
4L	574	73904	0.007779	2453	64091	0.03827	392.54 E
4R	4853	147741	0.03285	3129	192647	0.01624	102.28 V
5L	2688	138071	0.01947	3941	117496	0.03354	72.27 E
5R	2468	149023	0.01656	2671	124743	0.02141	29.29 V
6L	3338	140761	0.02371	1375	140738	0.00977	142.68 V
6R	2588	246118	0.01052	2312	74221	0.03115	196.10 E
7L	1901	147112	0.01292	2026	71140	0.02848	120.43 E
7R	3134	121533	0.02579	2088	76921	0.02714	5.23 V
8L	2451	80387	0.03049	3850	87558	0.04400	44.30 V
8R	3505	141312	0.02480	1851	121690	0.01521	63.05 E
9L	1407	69180	0.02034	5426	156424	0.03469	70.55 V
9R	1843	68418	0.02694	4032	118697	0.03397	26.10 E
10L	2847	113657	0.02505	4555	100002	0.04555	81.84 E
10R	2527	190141	0.01329	2574	139375	0.01847	38.98 V

TABLE 4.7 (continued)

	PROXIMAL			DISTAL				
NERVE	AXONS	AREA	DENSITY	AXONS	AREA	DENSITY	PERCENTAGE DIFFERENCE	
11L	1425	46258	0.03081	2615	111788	0.02340	31.17	E
11R	2998	673518	0.00445	2735	93951	0.02911	554.16	V
12L	1532	50073	0.03060	2658	58601	0.04536	48.24	V
12R	1528	96462	0.01584	3432	93743	0.03661	131.12	E
13L	3072	99550	0.03086	1351	118525	0.01140	170.70	V
13R	1190	78771	0.01511	2456	66516	0.03692	144.34	E
14L	2107	88381	0.02384	327	17069	0.01916	24.43	V
14R	1919	77558	0.02474	3562	112085	0.03178	28.46	E
15L	257	367000	0.00070	4312	137360	0.03139	3484.29	E
15R	1908	94935	0.02010	1645	103995	0.01582	27.05	V
16L	252	95234	0.00265	2748	109320	0.02514	848.68	E
16R	1940	80316	0.02415	2137	83542	0.02558	5.92	V
17L	3465	419572	0.00826	4058	99960	0.04060	391.53	V
17R	-	-	-	-	-	-	-	
18L	1205	745857	0.00162	3669	80572	0.04554	2711.11	E
18R	-	-	-	-	-	-	-	
19L	3680	138283	0.02661	2498	66273	0.03770	41.68	V
19R	1964	76275	0.02575	4329	115844	0.03737	45.13	E
20L	1998	90167	0.02216	3325	129329	0.02570	15.97	E
20R	2140	101115	0.02116	1807	48971	0.03690	74.39	V

Wilcoxon Signed Rank Sum Test is non-significant

Rat 17 and 18 were unsuitable for assessment

TABLE 4.8 - WILCOXON SIGNED RANK SUM TEST TO DETERMINE IF ETHILON SUTURE PRODUCES A DIFFERENT DENSITY OF AXONS ACROSS A NERVE ANASTOMOSIS THAN VICRYL

RAT	ETHILON-VICRYL PERCENTAGE	RANK	SIGNED RANK
1	-157.26	12	-12
2	361.41	14	14
3	1701	17	-17
4	290	13	13
5	42.98	6	6
6	54	8	8
7	115	11	11
8	19	3	3
9	-44.5	7	-7
10	42.86	5	5
11	-523	15	-15
12	82.88	10	10
13	-26	4	-4
14	4	2	2
15	3457	18	18
16	842.76	16	16
19	3.45	1	1
20	-58.42	9	-9

Rat 17 and 18 were unsuitable for assessment

Wilcoxon Signed Rank Sum Test is non-significant T(-) = 64

TABLE 4.9 - VICRYL VERSUS ETHILON END TO END ANASTOMOSIS. AN ASSESSMENT OF THE CROSS SECTIONAL AREA, IN SQUARE MILLIMETRES, AND QUALITY OF THE AXONS

	ETHILON					VICRYL				
Proximal										
N	647	643	697	922	916	914	850	651	721	709
x	6.05	5.26	6.1	5.5	5.56	5.75	5.85	6.17	5.04	5.63
SD	2.42	2.14	2.7	2.02	2.29	2.52	1.97	3.19	1.65	2.34
Distal										
N	626	783	779	716	612	741	732	655	740	777
x	5.26	5.15	6.62	5.44	7.7	5.47	5.56	6.57	5.62	5.55
SD	2.13	2.08	2.98	2.35	2.76	2.16	2.4	2.54	2.23	2.04

There is no significant difference in the size of the axons in either the Vicryl or Ethilon anastomosed nerves (Students t test).

APPENDIX 4:1

METHODS OF NERVE PREPARATION FOR SCANNING ELECTRON MICROSCOPY

The middle specimen was then taken for scanning electronmicroscopy. It was initially fixed in a 10% phosphate buffered formalin solution for 1 - 2 hours. Thereafter it was placed in a 1% osmium tetroxide solution in 0.1 M cacodylate buffer for 30 minutes. This is a lipid fixative of myelin and nuclei. The section was then washed in distilled water to remove any excess osmium and dehydrated through graded acetone from 20% through to 100%. It was then at a critical point dried to remove liquid from the specimen without causing artifacts because of changes in the surface tension. It was then mounted on a ½" aluminium specimen stub with Araldite. It was coated with approximately 10 nm of gold by diode sputtering. It was then examined at 7.5 kV in a Cambridge Instruments PLC stereoscan 250 Mark III. A qualitative analysis of the specimen was then made.

5. COMPARISON OF EPINEURIAL AND PERINEURIAL SUTURES

5.1 INTRODUCTION

The current standard technique of peripheral nerve repair is an epineurial repair. The surgical technique is the one that has been best standardised and best studied with regard to long term results and is presently the most frequently performed (Omer, 1980). The cut surface of the nerve ends should be perpendicular to the longitudinal axis of the nerve as it is extremely difficult to perform an oblique nerve suture. Based on his studies of intraneural anatomy (Sunderland, 1944 and 1953) was the first to recommend clinical use of a fascicular repair. The relevance of fascicular suturing on the facial nerve remains contentious. Agreement is lacking in spite of efforts to determine whether or not the facial nerve is spatially orientated in its extra axial course from the brain stem to the periphery as it is in the cortex and pontine nucleus. Clinical observations have been reported that supports spatial orientation of the facial nerve within the temporal bone and as it exits the stylomastoid foramen (May, 1973). Millesi (1977) felt that the parotid segment of the facial nerve trunk had a distinct spatial orientation and applied this knowledge to perform fascicular repairs and interfascicular grafts. Several investigators have, however, not found evidence to support this hypothesis (Sunderland, 1953; Harris, 1968; Sade, 1975; Thomander et al, 1982; Gacek and Radpour, 1982). It is likely there is some

degree of spatial orientation at the facialnerve trunk and fascicular grafting at this level may well have a place in surgery of the future.

This experiment was designed to compare epineurial and perineurial sutures of the rat facial nerve. This nerve was chosen as it has quite discrete fascicles to suture as opposed to the buccal division of the facial nerve. It would be incorrect to directly transpose the results of this experiment to the human situation of suturing the facial nerve trunk but it reflects, in general, the relative merits of fascicular versus epineurial repair of a peripheral nerve.

5.2 METHODS AND MATERIALS

Twenty live Sprague-Dawley rats were used for experimental purposes. They were between 7 and 8 weeks old and weighed between 200 and 300 gm. They were anaesthetised in a similar manner to that described in Chapter 3. The fur of the lower back was trimmed and a transverse curvilinear incision was made across the lower back to expose the gluteal muscles. Dissection exposed the sciatic nerve. The nerve was dissected free although the nerve is extremely loosely bound at this point. The nerve was photographed, electrophysiologically tested and divided in a similar manner to that described in Chapter 3. A similar assessment of a minimal nerve twitch and a maximal nerve twitch was made. In a random fashion this nerve was sutured under microscopic control using either Vicryl or Ethilon with either 3 epineurial sutures placed equidistant from each other or the 4 discrete fascicles were approximated to each other with one suture each followed by 3 epineurial sutures (Table 5.1). The nerve was photographed and the wound sutured with 6/0 prolene. After 10 weeks the animals were taken, anaesthetised and the wound reopened. The nerve was photographed, tested electrophysiologically and a segment of the nerve removed which included the anastomosis. The animals were then sacrificed. The nerve segments were then prepared for histological examination as described in Chapter 3. Histological results were assessed in the same way as described in Chapter 3.

5.3 RESULTS

Photography

The sizes of the nerves were compared preoperatively to ensure there was no discrepancy between each side (Wilcoxon Signed Rank Sum Test) (Table 3.2). There was no significant difference. The quality and size of the anastomosis were studied from the photographs but no significant difference was found between the materials used or the type of anastomosis (Wilcoxon Signed Rank Sum Test). At 10 weeks photography again failed to show any significant difference between the various combinations of repair (Wilcoxon Signed Rank Sum Test) (Table 3.2).

Electrophysiology

The minimal muscle twitch test failed to demonstrate either the absorbable or non-absorbable sutures or the epineurial repair or the perineurial repair as being superior. There was no significant difference between the various combinations using the maximal muscle twitch (Wilcoxon Signed Rank Sum Test) (Table 5.2).

Histological

The proximal and distal ends of the nerve segment were prepared as described in Chapter 3. The results, as shown in Figures 5.1 to 5.3, demonstrate there is no significant difference in the

FIGURE 5.1

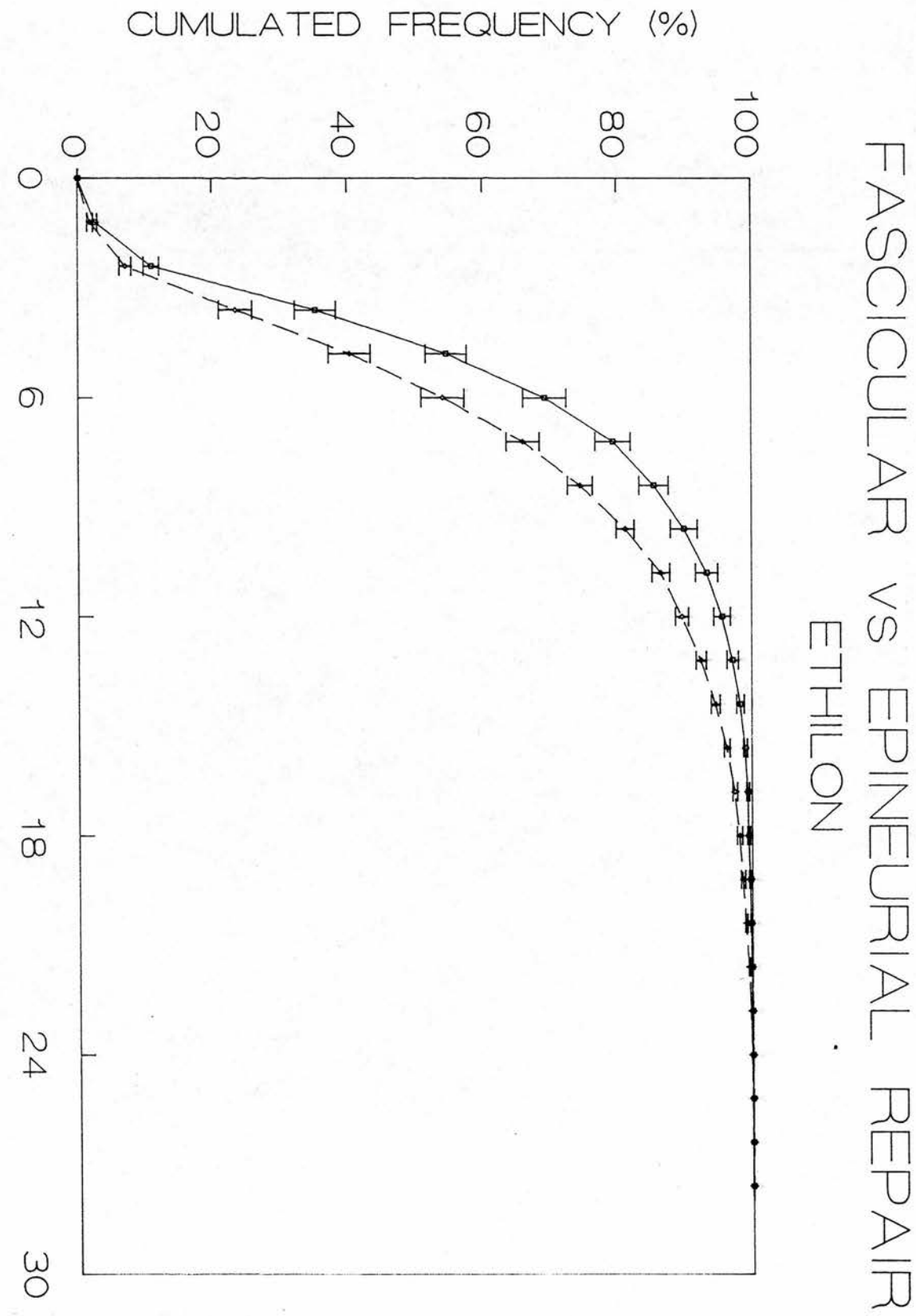

FASCICULAR VS EPINEURIAL REPAIR
ETHILON
CUMULATED FREQUENCY (%)
0
20
40
60
80
100
0
6
12
18
24
30
FIBRE DIAMETER (microns)

FIGURE 5.2

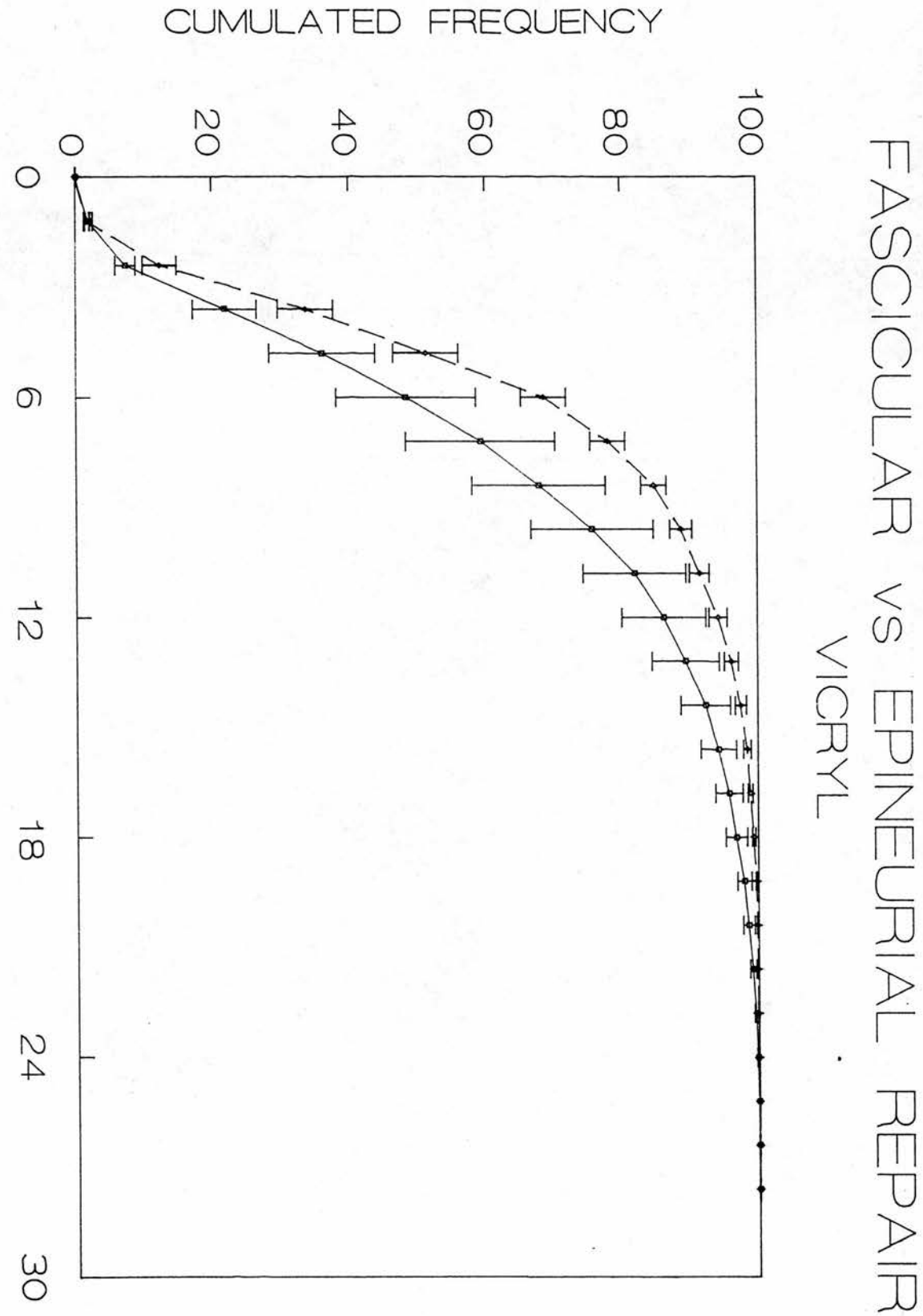
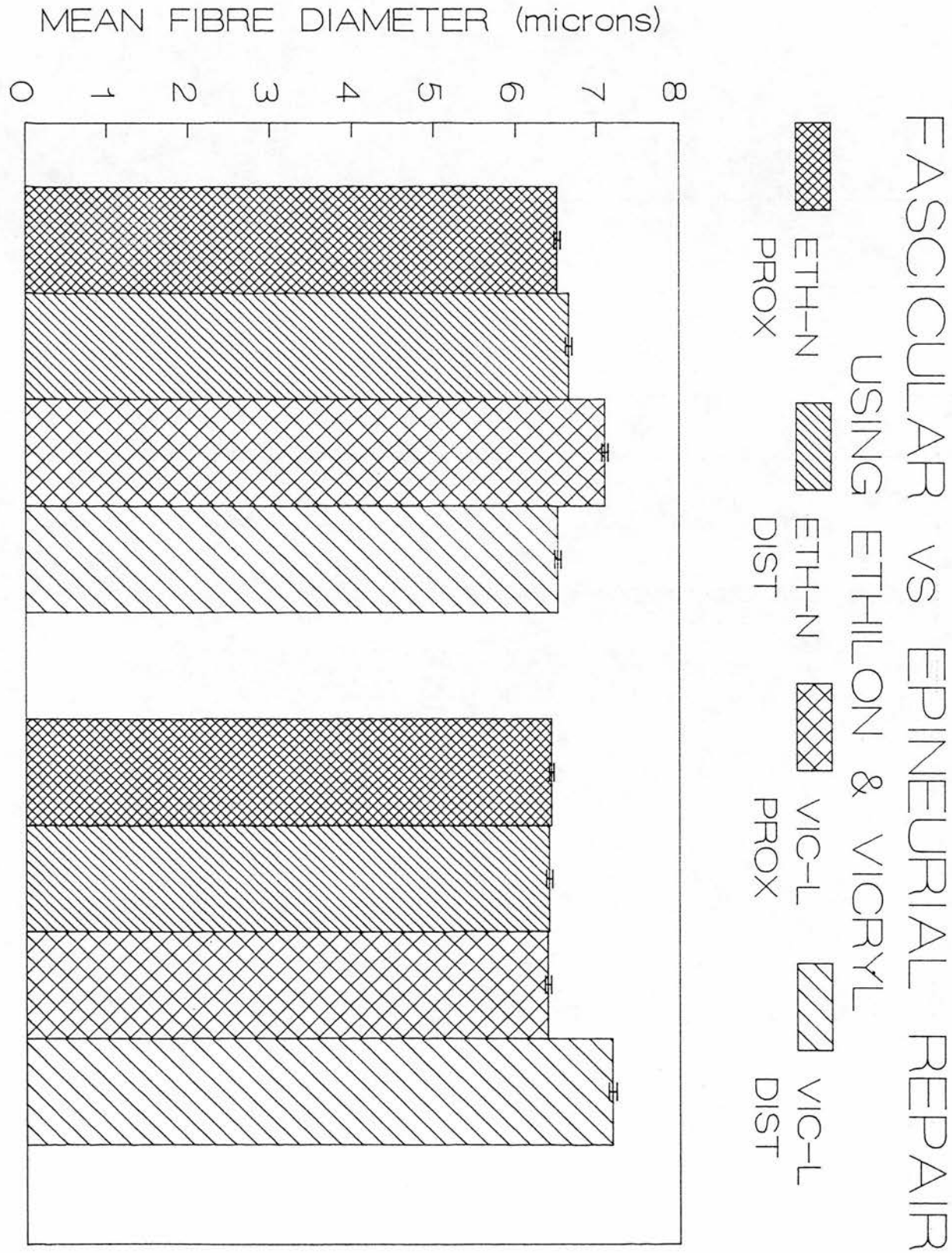

FASCICULAR VS EPINEURIAL REPAIR
VICRYL
CUMULATED FREQUENCY
0
20
40
60
80
100
0
6
12
18
24
30
FIBRE DIAMETER (microns)

FIGURE 5.3


FASCICULAR VS EPINEURIAL REPAIR
USING ETHILON & VICRYL
ETH-N PROX
ETH-N DIST
VIC-L PROX
VIC-L DIST
MEAN FIBRE DIAMETER (microns)
0
1
2
3
4
5
6
7
8
FASCICULAR
EPINEURIAL

quality of axons crossing the anastomosis. There is a suggestion that Vicryl produces slightly smaller axons with fascicular repair than with the epineurial repair but this is not significant ($0.5 > p > 0.1$ in Student's t test). Similarly Ethilon appears to produce slightly bigger axons with the perineurial repair compared with epineurial repair but this is not significant ($0.5 > p > 0.1$ in Student's t test) (Tables 5.3 and 5.4).

5.4 DISCUSSION

Epineurial repair of a divided peripheral nerve has been the traditional method of repair since the late 1800s (Heuter, 1873). However, a significant percentage of patients do not regain useful function (Moberg, 1964). Edsage (1964) showed this method could cause malalignment and buckling or displacement of fascicles. With some loss of nerve substance an epineurial repair could guide many axons into the epineurial tissues or incorrect distal endoneurial tubes - both leading to useless regeneration. Millesi et al (1967) argue that the epineurium is the site of scar tissue formation in the repair site and should be removed before repair. However, Kline et al (1981) concluded that there is no advantage in either resecting or closing the epineurium in the perineurial repair. The epineurium should not intrude between the endoneurium of the proximal and distal fascicles in any type of nerve repair. Individual fascicles are teased loose from each other. Only one suture per fascicle is necessary. There are inherent disadvantages of attempting to relate the results of experimental studies to the treatment of humans. These include different rates of nerve regeneration, dissimilar scarring potential and the simpler fascicular patterns found in lower animals.

Animal research studies have, however, unquestionably some advantage. These include the ability to closely control experimental techniques and to analyse the resultant data which is not

possible in humans. There appears to be no consensus in research literature as to which is the superior method of suture (Orgel, 1984; Tupper et al, 1988) as was found in the present experiment. Kline et al (1981) compared epineurial and perineurial repairs in multifascicular nerves in primates. They assessed the results by quantitative morphology and electrophysiology. They demonstrated conclusively that in non-human primates perineurial suture of a lacerated multifascicular nerve confers no advantage over the standard epineurial repair. It does seem reasonable that the epineurial and fascicular repairs may be used in specifically appropriate cases. If mixed fasciculi are present at the level of the lesion of the nerve, epineurial repair is preferable. Fascicular repair is more suitable when pure motor and sensory fasciculi are recognised. Epineurial repair is indicated for more proximal injuries and fascicular repair is appropriate for distal sites (Zhong et al, 1988).

Yamamoto (1988) compared epineurial and perineurial suturing of the orbicularis oculi branch of the facial nerve of cats. Nerve regeneration was slightly reduced with perineurial suture without a tube compared with epineurial suture with a tube, epineurial suture without a tube and perineurial suture with a tube. The point is made that if discrete corresponding fasciculi cannot be identified, an epineurial suture is sufficient. Possibly a more important factor in the end result of suture of the sciatic nerve is tension at the anastomosis which

is noticeably absent in the repair of the buccal nerve.

The epineurial sutures used in the fascicular repair in the experiment described above were used to avoid undue tension at the anastomosis. This technique was described by Fernandez and Pallini (1987) in the repair of 3 ulnar and 2 cranial nerve lesions.

5.5 CONCLUSION

There appears to be no significant difference in the outcome of anastomosing a divided rat sciatic nerve by either the epineurial or perineurial technique using absorbable sutures (Vicryl 10/0) or non-absorbable sutures (Ethilon 10/0) as assessed by photographic, electrophysiological or histological means.

TABLE 5:1 – FASCICULAR AND EPINEURIAL REPAIR OF THE DIVIDED RAT SCIATIC NERVE USING ETHILON AND VICRYL

	NERVE	ANASTOMOTIC AGENT	PROCEDURE
Rat 1.	Sciatic – LHS	Ethilon	Fascicular repair
	– RHS	Ethilon	Epineurial repair
Rat 2.	Sciatic – LHS	Ethilon	Epineurial repair
	– RHS	Ethilon	Fascicular repair
Rat 3.	Sciatic – LHS	Ethilon	Fascicular repair
	– RHS	Ethilon	Epineurial repair
Rat 4.	Sciatic – LHS	Ethilon	Epineurial repair
	– RHS	Ethilon	Fascicular repair
Rat 5.	Sciatic – LHS	Ethilon	Fascicular repair
	– RHS	Ethilon	Epineurial repair
Rat 6.	Sciatic – LHS	Vicryl	Fascicular repair
	– RHS	Vicryl	Epineurial repair
Rat 7.	Sciatic – LHS	Vicryl	Epineurial repair
	– RHS	Vicryl	Fascicular repair
Rat 8.	Sciatic – LHS	Vicryl	Fascicular repair
	– RHS	Vicryl	Epineurial repair
Rat 9.	Sciatic – LHS	Vicryl	Epineurial repair
	– RHS	Vicryl	Fascicular repair
Rat 10.	Sciatic – LHS	Vicryl	Fascicular repair
	– RHS	Vicryl	Epineurial repair

TABLE 5.1 (continued)

	NERVE	ANASTOMOTIC AGENT	PROCEDURE
Rat 11.	Sciatic - LHS	Ethilon	Fascicular repair
	- RHS	Ethilon	Epineurial repair
Rat 12.	Sciatic - LHS	Ethilon	Epineurial repair
	- RHS	Ethilon	Fascicular repair
Rat 13.	Sciatic - LHS	Ethilon	Fascicular repair
	- RHS	Ethilon	Epineurial repair
Rat 14.	Sciatic - LHS	Ethilon	Epineurial repair
	- RHS	Ethilon	Fascicular repair
Rat 15.	Sciatic - LHS	Ethilon	Fascicular repair
	- RHS	Ethilon	Epineurial repair
Rat 16.	Sciatic - LHS	Vicryl	Fascicular repair
	- RHS	Vicryl	Epineurial repair
Rat 17.	Sciatic - LHS	Vicryl	Epineurial repair
	- RHS	Vicryl	Fascicular repair
Rat 18.	Sciatic - LHS	Vicryl	Fascicular repair
	- RHS	Vicryl	Epineurial repair
Rat 19.	Sciatic - LHS	Vicryl	Epineurial repair
	- RHS	Vicryl	Fascicular repair
Rat 20.	Sciatic - LHS	Vicryl	Fascicular repair
	- RHS	Vicryl	Epineurial repair

TABLE 5.2 - AN ELECTROPHYSIOLOGICAL COMPARISION OF THE EFFECT OF PERINEURIAL AND EPINEURIAL REPAIR USING AN ABSORBABLE AND NON ABSORBABLE SUTURE IN THE REPAIR OF A DISSECTED RAT SCIATIC NERVE (MINIMAL AND MAXIMAL EXCITABILITY TEST)

		STUDENT'S 'T' TEST
Ethilon	Perineurial versus epineurial	Minimal $0.5 > p > 0.1$
		Maximal $0.5 > p > 0.1$
Vicryl	Perineurial versus epineurial	Minimal $0.5 > p > 0.1$
		Maximal $0.5 > p > 0.1$

TABLE 5.3 - FASCICULAR AND EPINEURIAL REPAIR OF SCIATIC NERVE USING VICRYL AND ETHILON. A STATISTICAL COMPARISON OF THE CROSS SECTIONAL AREA, IN MILLIMETERS SQUARE, AND THE QUALITY OF THE AXONS ACROSS THE ANASTOMOSIS.

	ETHILON		VICRYL	
	FASCICULAR	EPINEURIAL	FASCICULAR	EPINEURIAL
Proximal				
x	6.51	6.43	7.09	6.38
SD	2.97	2.91	3.33	3.09
SEM	0.04	0.03	0.04	0.04
N	6689	7246	6600	6726
Distal				
x	6.65	6.40	6.51	7.17
SD	3.03	3.09	3.02	3.46
SEM	0.04	0.04	0.04	0.05
N	6895	5942	6399	5216

There is no statistical difference between the 2 anastomotic materials or the 2 different techniques of repair (Student's t test) (Table 5.4)

TABLE 5.4 - EPINEURIAL VERSUS PERINEURIAL REPAIR

		STUDENTS 'T' TEST
Proximal		
Ethilon	Fascicular versus epineurial	$0.5 > p > 0.1$
Vicryl	Fascicular versus epineurial	$0.5 > p > 0.1$
Distal		
Ethilon	Fascicular versus epineurial	$0.5 > p > 0.1$
Vicryl	Fascicular versus epineurial	$0.5 > p > 0.1$
Ethilon		
Fascicular	Proximal versus distal	$0.5 > p > 0.1$
Epineurial	Proximal versus distal	$0.5 > p > 0.1$
Vicryl		
Fascicular	Proximal versus distal	$0.5 > p > 0.1$
Epineurial	Proximal versus distal	$0.5 > p > 0.1$

6. A COMPARISON OF GLUE AND A TUBE AS AN ANASTOMOTIC AGENT TO REPAIR THE DIVIDED BUCCAL BRANCH OF THE RAT FACIAL NERVE

6.1 INTRODUCTION

Alternatives to suture materials to anastomose small nerves have been sought for many years. Lyons and Petrocelli (1978) describe how, in ancient Greece, plant resins were used to repair wounds and the ancient Egyptians used an adhesive vegetable gum for this purpose. After the discovery of cyano-acrylates in the mid 20th century, Siedentop and Loewy (1979) compared anastomosis with suture and stabilisation with cyano-acrylate in the facial nerve of 13 dogs. They concluded the histological results of both techniques were similar. Unfortunately, the histotoxic effects of the glue became subsequently apparent (Vinters et al, 1985). Parker et al (1984) compared a mononylon suture anastomosis with haemostatic microfibrillar collagen anastomosis in the extratemporal portion of the facial nerve of the rabbit. The clinical results were identical but histologically the nylon suture appeared less reactive. Szal and Miller (1975) noted the best results of facial nerve anastomosis in rabbits were from epineurial suture compared with vein wraps or sialastic. In contrast, Beodts and Bouckaert (1984) described an experiment comparing various sutures and fibrin glue to repair the divided sciatic nerve of the rat. They found superior results from the glue. Becker et al (1985) compared epineurial suture and fibrin glue to repair

the divided sciatic nerve of the rat but found no difference. There, thus, appears to be considerable controversy over the use of the fibrin glue for nerve anastomosis. Similarly the use of a 'tube wrap' around the anastomosis has the theoretical advantage of stabilising the nerve ends and decreasing the number of extra epineurial regenerating axons but many of the substances used for the wrap have given rise to problems in their own right. In this experiment, the use of a fibrin glue was compared with a tube of collagen around the site of anastomosis of the buccal nerve in rats.

6.2 METHODS AND MATERIALS

Twenty live Sprague-Dawley rats were used. They were 6 to 7 weeks old and weighed between 200 and 300 gm. They were prepared as detailed in Chapter 3 (Table 6.1). The buccal division of the facial nerve was exposed, photographed, electrically stimulated, divided and anastomosed either with Tisseel, human fibrin glue and the ends laid together or a collagen wrap around the anastomosis to form a tube was fashioned and the end result photographed. Ten weeks later the buccal nerves were reexposed, photographed and tested electrophysiologically. The anastomosis was photographed and the nerve segment removed 1 cm proximal and distal to the anastomosis. The presection photographs were quantitatively assessed to ensure that the nerves on both sides were of a similar size. The pictures of the newly made anastomosis were assessed qualitatively to determine if there was a differences in the quality of anastomosis. The pictures of the 10 week old anastomosis were assessed qualitatively and quantitatively for any reduction in the transverse diameter of the nerve and the quality of the end result of the anastomosis. The nerve was tested electrophysiologically in a similar manner as detailed in Chapter 3. The nerve segment was histologically assessed as detailed in Chapter 3.

6.3 RESULTS

Photography

There was no significant difference in the transverse size of the preoperative buccal nerves, comparing one side with the other. There was no significant difference in the macroscopic quality of the anastomosis between the glued ends and the wrapped ends and there was no significant difference in either the quality of the anatomosis or the transverse size of the proximal and distal ends of the nerves 10 weeks after the anastomosis (Table 3.2).

Electrophysiology

The minimal excitability tests failed to show any significant difference between the 2 techniques of anastomosis using the Wilcoxon Signed Rank Sum Test. Similarly, the maximal excitability test did not show any significant difference between the 2 techniques of anastomosis using the Wilcoxon Signed Rank Sum Test (Table 6.2).

Histology

Figures 6.1 and 6.2 summarise the results (Tables 6.3 and 6.4). The quality of axons in the nerve are similar when the glue or collagen wrap are used for the anastomosis. There is no

FIGURE 6.1

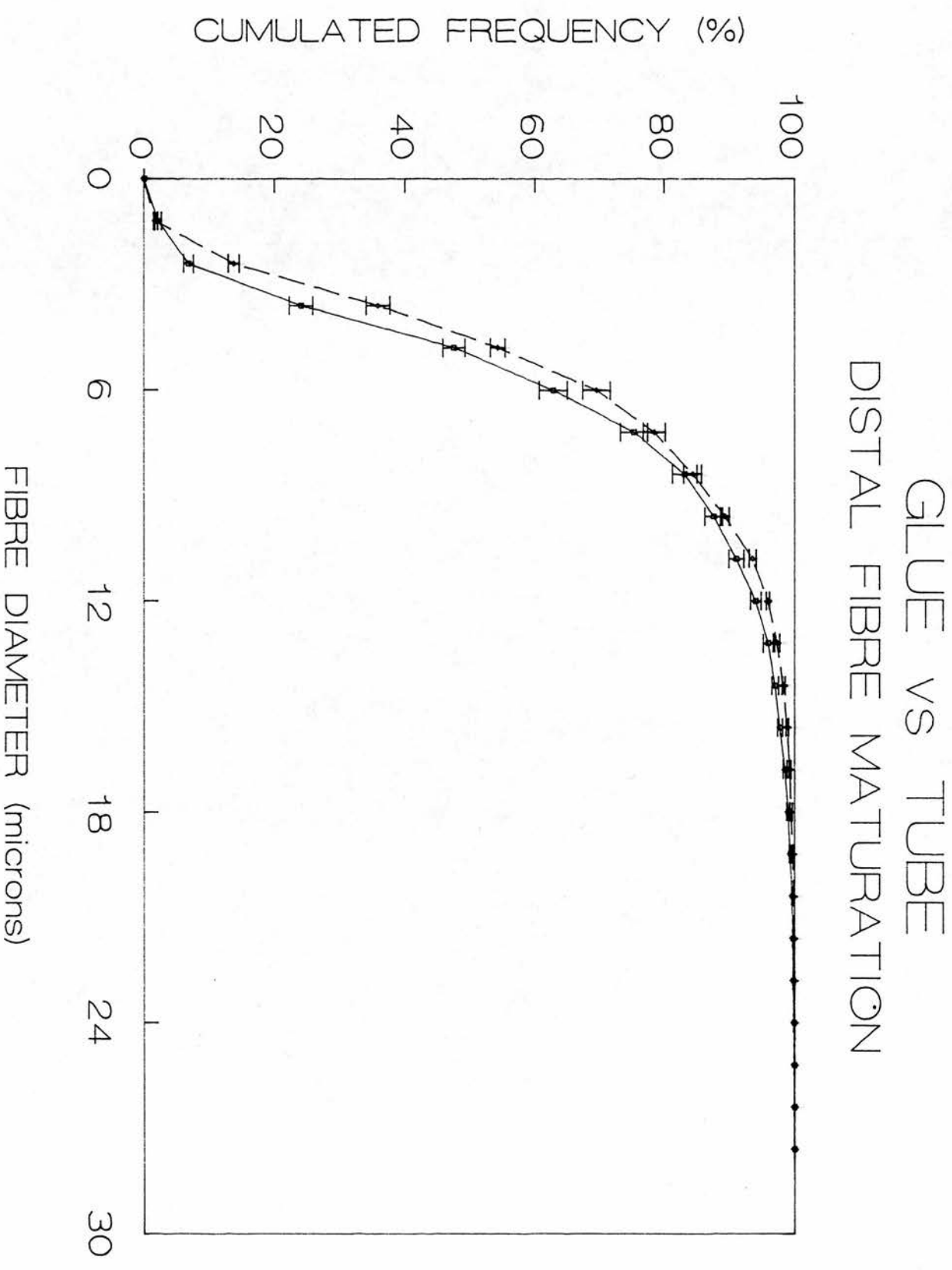
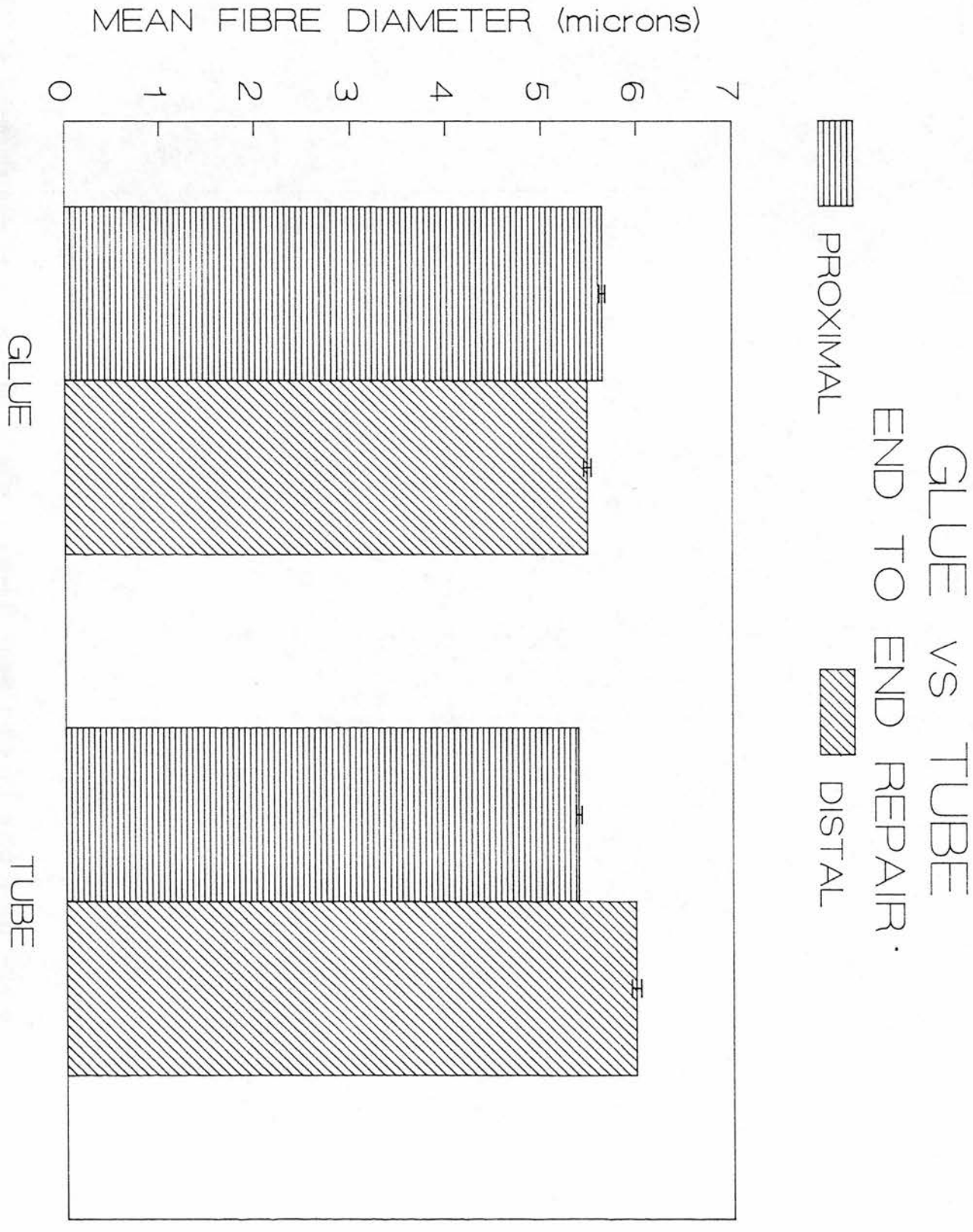

GLUE VS TUBE
DISTAL FIBRE MATURATION
CUMULATED FREQUENCY (%)
0
20
40
60
80
100
0
6
12
18
24
30
FIBRE DIAMETER (microns)

FIGURE 6.2


GLUE VS TUBE
END TO END REPAIR.
PROXIMAL
DISTAL
MEAN FIBRE DIAMETER (microns)
0
1
2
3
4
5
6
7
GLUE
TUBE

significant difference between the 2. Similarly there appears to be no fall off of axons across the anastomosis of a significant difference, ie there is no significant difference between the quality of axons before the anastomosis as after the anastomosis, indicating that the anastomotic site has probably matured and not filled with multiple immature axons which either fail to cross the anastomosis or subsequently die off. There is no significant difference between using tubes or glue as the anastomotic agent on the functioning axons in either the distal or proximal nerve ends (Students 't' test).

6.4 DISCUSSION

The choice of Tisseel glue, as opposed to other glues, was deliberate. The human fibrin glue is available commercially for specific research projects, it is easy to use and does not stimulate any antigenic reaction in the rat. The latter fact was confirmed in the control experiments in Chapter 3. The collagen wrap was also chosen deliberately. The collagen was initially tested in rats (described in Chapter 3) to ensure there was no antigenic or untoward reaction and it was absorbed by the time the anastomosis had matured. Fisch et al (1987) used fenestrated collagen splints in 8 patients operated on for an acoustic neuroma or glomus tumour. They report the technique for anastomosing the facial nerve at the cerebellar-pontine angle is rapid and simpler than suturing at this site. The clinical results were as good as, or better than, using conventional suturing techniques. Hamm et al (1987) found that in rat experiments the microsurgical suture technique provided better axon regeneration powers than using the fibrin glue technique. The latter technique was as good as suture if a nerve transplant was used. Boedts (1987) points out that the use of fibrinogen - thrombin adhesive to seal nerve ends is an easy, time saving method at surgery and provides excellent coaptation of the severed nerve fascicles with minimal iatrogenic trauma. There remains the problem of excessive connective tissue proliferation at the junction site or of premature loss of tensile strength before proper nerve healing occurs. He suggests a combination

of gluing the nerve ends and the tube is the ideal anastomotic agent. Feldman et al (1987) repaired the transected sciatic nerve of rabbits with either an autologous fibrin-based glue or conventional perineurial suturing. Both techniques showed similar results in functional evaluation and histology, comparing anastomotic fibrosis, axonal regeneration and fascicular alignment. Smahel et al (1987) carried out a similar study on the rat sciatic nerve. They used tissucol glue and compared this with epineurial sutures. The fibrin glue was a simpler method with no distraction of the nerve ends. There was no long term differences in electrophysiological or histological results.

Moy et al (1988) compared the results of repair of the tibial nerve of New Zealand rabbits with either a fibrin seal or a 10/0 monofilament, non-absorbable nylon suture. They comment on the fact that the repair takes significantly ($p < 0.01$) less time using the fibrin seal. The results, using the fibrin seal, were poor from the electrophysiological and histological criteria. Narakas (1988) estimated that by using a glue instead of a suture, the operating time to repair a nerve is decreased to 30% of the time needed using a conventional suture technique. He feels in the human situation, the percentage of good results is increased by about 15%. Cruz et al (1986) evaluated the merits of homologous fibrin glue in the repair of peripheral nerve transections as compared to standard epineurial suture repairs in rats. In 80% of glued ends of the sciatic nerve, a dehiscence of the anastomosis occurred and he felt that the glue

merely increased the inflammatory reaction if used in conjunction with a 2-suture repair. Medders et al (1989) tested the effect of fibrin glue on nerve regeneration on rats. Nerve repairs were performed with and without fibrin glue on the intratemporal facial nerve. On the experimental side the nerve was repaired with fibrin glue and on the control side the nerve was reapproximated in the Fallopian canal without glue or suture. Axon counts distal to the repair showed no statistically significant difference between the two methods of repair. This suggested that mechanical obstruction by the fibrin glue between the nerve ends has a negligible effect on nerve regeneration.

Like most authors, they counted the number of axons rather than studying the size and not the maturity of the axons which is a better indication of the efficacy of the repair.

Nishihira and McCaffrey (1989) assessed the equality of nerve repair in the sciatic nerve of the rat in a comparison of simple and autogenous nerve graft using microsurgical sutures and fibrin glue. There was essentially no difference in these repairs.

Calteux et al (1984) used a venous sleeve as a tube around the nerve anastomosis in dogs. The found that histologically and electrophysiologically the repair was better than using other conventional anastomotic techniques. Rosen et al (1983) used a polyglycolic acid tube to isolate the discrete fascicles of rats'

peripheral nerves as a method of anastomosis. They feel this decreases connective tissue intervention in the anastomosis and claim there is improved organisation of the repair site compared with suture repair. The polyglycolic acid tube is reabsorbed after the perineurium has reestablished its continuity. Kulgis et al (1983) compared the results in the repair of divided sciatic nerves of rats using bilateral fascicular neurrohaphy and a dura mater sheet wrap on one side. The cuffed side showed a longer extent of retrograde myelin and axonal degeneration, a faster rate of orthograde remyelination, axonal invasion of the suture plane at about the same period (fifth postoperative day), a larger contingent of regenerating fibres invading the distal stump, more longitudinally orientated fibres at the repair level, no escape of fibres into the extraneural tissue through the repair and less intra-neural oedema.

Henry et al (1985) studied the effects of biodegradable polyester tubes of various diameters on the regeneration of transected peripheral nerves in mice. They concluded the more biodegradable the tube, the more likely it was to cause distortion and lumenal narrowing. Lumenal adequacy, not the tube composition, was important in nerve regeneration. The larger the lumen relative to the size of the nerve, the better was axonal regeneration. Bento (1988) found that intratemporal anastomosis of the facial nerve in cats was technically much easier with glue than suture and the functional results were similar. Both these methods were superior to merely laying the 2 ends together.

6.5 CONCLUSION

There appears to be no significant difference in the outcome of anastomosing the divided buccal division of the rat facial nerve using a glue method (Tisseel) and a tube wrap method (collagen wrap) as assessed by photographic, electrophysiological or histological means.

TABLE 6.1 - NERVE ANASTOMOTIC AGENT AND PROCEDURE

	NERVE	ANASTOMOTIC AGENT	PROCEDURE
Rat 41.	Facial - LHS	Tube	Nerve cut and repaired
	- RHS	Glue	Nerve cut and repaired
Rat 42.	Facial - LHS	Glue	Nerve cut and repaired
	- RHS	Tube	Nerve cut and repaired
Rat 43.	Facial - LHS	Tube	Nerve cut and repaired
	- RHS	Glue	Nerve cut and repaired
Rat 44.	Facial - LHS	Glue	Nerve cut and repaired
	- RHS	Tube	Nerve cut and repaired
Rat 45.	Facial - LHS	Tube	Nerve cut and repaired
	- RHS	Glue	Nerve cut and repaired
Rat 46.	Facial - LHS	Glue	Nerve cut and repaired
	- RHS	Tube	Nerve cut and repaired
Rat 47.	Facial - LHS	Tube	Nerve cut and rapaired
	- RHS	Glue	Nerve cut and repaired
Rat 48.	Facial - LHS	Glue	Nerve cut and repaired
	- RHS	Tube	Nerve cut and repaired
Rat 49.	Facial - LHS	Tube	Nerve cut and repaired
	- RHS	Glue	Nerve cut and repaired
Rat 50.	Facial - LHS	Glue	Nerve cut and repaired
	- RHS	Tube	Nerve cut and repaired

TABLE 6.1 (Continued)

	NERVE	ANASTOMOTIC AGENT	PROCEDURE
Rat 56.	Facial - LHS	Tube	Nerve cut and repaired
	- RHS	Glue	Nerve cut and repaired
Rat 57.	Facial - LHS	Glue	Nerve cut and repaired
	- RHS	Tube	Nerve cut and repaired
Rat 58.	Facial - LHS	Tube	Nerve cut and repaired
	- RHS	Glue	Nerve cut and repaired
Rat 59.	Facial - LHS	Glue	Nerve cut and repaired
	- RHS	Tube	Nerve cut and repaired
Rat 60.	Facial - LHS	Tube	Nerve cut and repaired
	- RHS	Glue	Nerve cut and repaired
Rat 61.	Facial - LHS	Glue	Nerve cut and repaired
	- RHS	Tube	Nerve cut and repaired
Rat 62.	Facial - LHS	Tube	Nerve cut and repaired
	- RHS	Glue	Nerve cut and repaired
Rat 63.	Facial - LHS	Glue	Nerve cut and repaired
	- RHS	Tube	Nerve cut and repaired
Rat 64.	Facial - LHS	Tube	Nerve cut and repaired
	- RHS	Glue	Nerve cut and repaired
Rat 65.	Facial - LHS	Glue	Nerve cut and repaired
	- RHS	Tube	Nerve cut and repaired

TABLE 6.2 - AN ELECTROPHYSIOLOGICAL COMPARISON OF THE EFFECT OF A COLLAGEN TUBE AND GLUE REPAIR OF A DISSECTED BUCCAL DIVISION OF THE FACIAL NERVE OF A RAT

	STUDENT'S 'T' TEST
Tube	Minimal $0.5 > p > 0.1$
	Maximal $0.5 > p > 0.1$
Glue	Minimal $0.5 > p > 0.1$
	Maximal $0.5 > p > 0.1$

TABLE 6.3 - STUDENTS 'T' TEST APPLIED TO COMPARE THE QUALITY OF AXONS FROM A TUBE OR GLUE ANASTOMOSIS

		STUDENTS 'T' TEST
Proximal	Tube versus glue	$0.5 > p > 0.1$
Distal	Tube versus glue	$0.5 > p > 0.1$
Tube	Proximal versus distal	$0.5 > p > 0.1$
Glue	Proximal versus distal	$0.5 > p > 0.1$

TABLE 6.4 - A COMPARISON OF THE QUALITY AND CROSS SECTIONAL AREA, IN SQUARE MILLIMETRES, OF AXONS FROM A TUBE OR GLUE ANASTOMOSIS

	TUBE	GLUE
Proximal		
x	5.38	5.64
SD	2.10	2.35
SEM	0.03	0.03
N	6000	6563
Distal		
x	5.98	5.48
SD	2.62	2.24
SEM	0.05	0.04
N	3358	3338

7. GENERAL DISCUSSION

The buccal division of the rat facial nerve is an excellent nerve to use as an animal model. There may be some criticisms levelled at using rats and the translation of any work from rats into human facial nerves but, nevertheless, the animal model is valid to a limited extent.

This experiment shows that the materials used, ie absorbable, non-absorbable, a collagen tube and a plasma glue are inert to the nerve and allow natural healing of a divided facial nerve with minimal influence from the materials themselves. This would certainly suggest that the discussion over exactly what material should be used on the grounds of interference with axon regrowth should now be discounted and more attention paid to the practical details of the site of nerve section. It is suggested if the site allows the nerves to lie together without a movement, eg within the temporal bone, then probably the glue or tube would be appropriate, particularly as this area may be difficult to suture, whereas in the extratemporal portion of the facial nerve where there is potential for more movement of the face, a suture material may be appropriate. There appears to be no difference in the end results of the different types of repair to the facial nerve itself.

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STATISTICAL APPENDIX

This appendix has been written to more fully explain the results shown in the original ChM volume. I have relied heavily on Dr Robin Prescott's (department of medical statistics and computing, University of Edinburgh) advice and guidance regarding the statistics and all the statistical tests were applied to his direction.

VALIDATION OF STATISTICAL METHODS

The Choice of the Statistical Test

In all of the experiments one method of anastomosis of the divided rat facial or sciatic nerve was compared with another on the effects of the material on the intact nerve were compared. The results were assessed photographically, electrophysiologically and histologically.

In each group the numbers were small. There was no evidence to suggest before the experiments that there was a 'normal' distribution of results. For both these reasons, the initial statistical analysis would require to be a non-parametric test. The histological analysis requires further explanation. The raw data of counting axons alone should be subject to a non-parametric test because the axons are of considerably different sizes and, in the processing, some may become deformed but still countable. In Chapter 4 of the ChM thesis, these raw data are shown. However it was decided, that for practical purposes only, those good quality large axons which innervate the distal nerve were of reasonable function and worth study. In Chapters 3, 4, 5 and 6, therefore, in the form of graphs and bar charts, the results of the measurement of quality of axons as well as numbers are shown. These reasonable quality axons have a normal distribution, hence parametric tests are applied.

The Wilcoxon Rank Sum Test was applied throughout the thesis. This, however, gives a conservative result. Kendal's Rank Correlation Test

was reapplied. These tests may measure subtly different aspects, eg the rank correlation test may measure the proportional change of axons across the anastomosis and Wilcoxon Rank Sum Test the magnitude of the change.

It has been suggested that a 2 way analysis of variance should be applied as a statistical test specifically for Table 4.5. There was, in fact, a mathematical error in the first attempt at the Wilcoxon Rank Sum Test because some of the signs (+ or −) were inadvertantly omitted leading to an erroneous result. The table has now been corrected using the Rank Correlation Test. This shows that some of the results were significant but the 2 way analysis of variance shows even greater significance. Therein lies the problem of statistics with slightly varying answers of significance depending on which test is applied. Data that fail to yield a significant result when subjected to simple tests but do so after a refined and complex analysis should be looked at critically. Success may be due to the use of more information by the complex method rather than the simple method but it may depend on the existence of differences that have no practical value. Also, statistical significance does not necessarily imply clinical significance. The methods generally used in this thesis represent simple statistical analyses which are designed to provide the most conclusive results for application in practice. It must also be borne in mind that one spurious result of significance in a mass of data must be regarded with common sense and not be held as success of a certain experiment if no other data support it.

TABLE 3.6a - A COMPARISON OF THE TRANSVERSE DIAMETER OF THE NERVE PROXIMAL AND DISTAL TO THE ANASTOMOSIS RELATIVE TO THE TRANSVERSE DIAMETER PREOPERATIVELY.

	PREOPERATIVE (mm)		POSTOPERATIVE (mm)	
RAT	PROX TD	DIST TD	PROX TD	DIST TD
Ethilon vs No Suture (Nerve Cut)				
1L	75	70	70	65
1R	70	60	75	70
2L	65	65	70	65
2R	80	80	75	80
3L	85	80	80	75
3R	60	65	65	60
4L	75	70	70	75
4R	85	80	85	75
5L	65	65	60	55
5R	70	75	65	55
Vicryl vs No Suture (Nerve Cut)				
6L	40	40	50	45
6R	45	40	50	50
7L	100	80	80	80
7R	90	80	100	80
8L	60	60	65	55
8R	65	60	70	30
9L	70	70	65	60
9R	80	70	70	60
10L	65	55	70	50
10R	70	65	75	60

TABLE 3.6a (Continued)

RAT	PREOPERATIVE (mm) PROX TD	DIST TD	POSTOPERATIVE (mm) PROX TD	DIST TD
Glue vs No Glue (Nerve Cut)				
11L	80	80	100	80
11R	90	90	100	85
12L	55	50	85	70
12R	60	65	70	65
13L	40	40	50	40
13R	50	45	45	40
14L	55	50	65	50
14R	65	65	70	50
15L	60	60	70	55
15R	80	80	70	65
Tube vs No Tube (Nerve Cut)				
16L	75	80	80	60
16R	65	60	70	70
17L	80	85	85	75
17R	90	80	90	90
18L	45	50	60	55
18R	40	40	40	40
19L	75	70	70	70
19R	65	70	60	55
20L	80	75	70	85
20R	90	85	90	85

TABLE 3.6a (Continued)

RAT	PREOPERATIVE (mm) PROX TD	PREOPERATIVE (mm) DIST TD	POSTOPERATIVE (mm) PROX TD	POSTOPERATIVE (mm) DIST TD
Ethilon vs Vicryl (Laid Through Nerve)				
21L	65	65	65	60
21R	70	75	70	70
22L	40	40	45	40
22R	45	50	45	50
23L	40	45	45	40
23R	45	45	50	40
24L	60	65	55	65
24R	60	60	60	60
25L	80	75	80	80
25R	80	80	75	75
Glue vs Tube (Laid Beside Nerve)				
26L	100	70	80	90
26R	80	60	90	65
27L	80	70	80	80
27R	65	60	60	65
28L	80	80	80	65
28R	50	50	80	80
29L	60	60	65	60
29R	55	60	60	55
30L	90	90	85	60
30R	80	80	90	70

TABLE 3.6b - RESULTANT DIFFERENCES FOR EACH MATERIAL

RAT	ETHILON	RAT	NO SUTURE
1L	0	1R	-5
2R	+5	2L	-5
3L	0	3R	-10
4R	-5	4L	+10
5R	-10	5L	-5

A rank correlation test for tied pairs was applied to these figures thus:

Ethilon: 5R, 4R, 1L, 2R 3L

No Suture: 3R, 1R, 4L 2R 5L

Ethilon 1, 2, 3½, 3½, 5

No Suture 3, 5, 3, 1, 3

$P = 1 + 0 + 1 + 0 = 2$ $\quad T = \frac{1}{2}\ 2\ (2 - 1) = 1$

$Q = 1 + 2 + 1 + 0 = 4$ $\quad u = \frac{1}{2}\ 3\ (3 - 1) = 3$

$$r = \frac{P - Q}{\frac{1}{2}\ u\ (u - 1) - T \qquad \frac{1}{2}\ n\ (n - 1) - u}$$

$$r = \frac{-2}{\frac{1}{2}\ 5\ (4) - 1 \quad \frac{1}{2}\ 5\ (4 - 3)}$$

$= -0.25$ Non significant

TABLE 3.6b (Continued)

RAT	VICRYL	RAT	NO SUTURE
6L	-5	6R	+5
7R	-10	7L	+20
8L	-10	8R	-35
9R	0	9L	-5
10L	-10	10R	-10

P = 5 Q = 5

r = 0 Non significant

RAT	GLUE	RAT	NO GLUE
11L	-20	11R	-15
12R	-10	12L	-10
13L	-10	13R	0
14R	-20	14L	-10
15L	-5	15R	-5

P = 7 Q = 2 T = 2 u = 1

r = 0.59 Non significant

RAT	TUBE	RAT	NO TUBE
16L	+25	16R	+5
17R	+10	17L	-15
18L	-10	18R	0
19R	-10	19L	+5
20L	+20	20R	0

P = 5 Q = 3 T = 2 u = 2

r = 0.24 Non significant

TABLE 3.6b (Continued)

RAT	ETHILON LAID THROUGH NERVE	RAT	VICRYL LAID THROUGH NERVE
21L	-5	21R	-5
22R	0	22L	+5
23L	-10	23R	-10
24R	0	24L	+5
25L	+5	25R	0

P = 6 Q = 2 T = 1 u = 1

r = 0.44 Non significant

RAT	GLUE NEXT TO NERVE	RAT	TUBE NEXT TO NERVE
26L	+40	26R	-5
27R	+10	27L	+10
28L	-15	28R	0
29R	-10	29L	-5
30R	-20	30L	-25

P = 6 Q = 3 T = 0 u = 1

r = 0.32 Non significant

TABLE 3.7 - MINIMAL EXCITABILITY TEST

RAT	ETHILON DIFFS	NO SUTURE DIFFS
1	2	-4
2	7	6
3	20	10
4	-2	8
5	5	-3

Rank Correlation Test

r = 0.4 Non significant

RAT	VICRYL DIFFS	VS	NO SUTURE DIFFS
6	5		6
7	-4		8
8	9		7
9	-11		-15
10	21		20

Rank Correlation Test

r = 0.2 Non significant

RAT	GLUE DIFFS	VS	NO SUTURE DIFFS
11	18		-6
12	-1		-3
13	0		1
14	7		7
15	3		9

Rank Correlation Test

r = 0 Non significant

TABLE 3.7 (Continued)

RAT	TUBE DIFFS	VS	NO SUTURE DIFFS
16	6		15
17	12		11
18	14		18
19	16		1
20	-2		5

Rank Correlation Test

r = 0.1 Non significant

RAT	ETHILON DIFFS	VS	VICRYL DIFFS NERVE INTACT
21	8		1
22	2		0
23	-5		6
24	7		2
25	0		0

Rank Correlation Test

r = 0.1 Non significant

RAT	GLUE DIFFS	VS	TUBE DIFFS NERVE INTACT
26	-6		18
27	-2		1
28	12		-4
29	1		6
30	5		-9

Rank Correlation Test

r = 0.4 Non significant

TABLE 3.8 - MAXIMAL EXCITABILITY TEST

RAT	ETHILON DIFFS	NO SUTURE DIFFS
1	5	-6
2	0	10
3	20	1
4	6	4
5	4	3

Rank Correlation Test

r = -0.4 Non significant

RAT	VICRYL DIFFS	NO SUTURE DIFFS
6	-30	-35
7	0	2
8	11	-8
9	14	20
10	2	-5

Rank Correlation Test

r = 0.4 Non significant

RAT	GLUE DIFFS	NO SUTURE DIFFS
11	21	12
12	8	18
13	16	1
14	30	16
15	1	-9

Rank Correlation Test

r = 0.2 Non significant

TABLE 3.8 (Continued)

RAT	TUBE DIFFS	NO SUTURE DIFFS
16	8	8
17	-5	12
18	6	15
19	9	1
20	2	5

Rank Correlation Test

r = -0.4 Non significant

RAT	ETHILON DIFFS	VICRYL DIFFS NERVE INTACT
21	30	6
22	3	-4
23	-24	-15
24	8	12
25	12	6

Rank Correlation Test

r = 0.42 Non significant

RAT	GLUE DIFFS	TUBE DIFFS NERVE INTACT
26	9	15
27	6	8
28	-5	2
29	14	10
30	11	12

Rank Correlation Test

r = 0.4 Non significant

TABLE 3.9 - OBSERVER SCORES FOR SEM OF ANASTOMOSED NERVE USING ETHILON AND VICRYL SUTURES AND CONTROLS (10 = PERFECT, 0 = WORST POSSIBLE)

RAT	SCORE (ETHILON)	RAT	SCORE (NO SUTURE)
1L	8	1R	2
2R	3	2L	9
3L	6	3R	1
4R	1	4L	5
5L	7	5R	3

P = 3 Q = 7

r = 0.4 Non significant

RAT	SCORE (VICRYL)	RAT	SCORE (NO SUTURE)
6L	2	6R	6
7R	5	7L	9
8L	3	8R	1
9R	9	9L	1
10L	4	10R	8

P = 5 Q = 4 T = 0 u = 1

r = 0.1 Non significant

TABLE 4.1 - A COMPARISON OF THE TRANSVERSE DIAMETER OF THE NERVE PROXIMAL AND DISTAL TO THE ANASTOMOSIS RELATIVE TO THE TRANSVERSE DIAMETER PREOPERATIVELY

	PREOPERATIVE			POSTOPERATIVE					
						RESULT		ETHILON	SIGNED
RAT	P TD	D TD	DIFF	P DIA	D DIA	DIFF	SUTURE	-VICRYL	RANK
1L	70 mm	70 mm	0	70 mm	60 mm	10	E	-10	-7
1R	50 mm	50 mm	0	80 mm	60 mm	20	V		
2L	80 mm	70 mm	10	115 mm	80 mm	25	V	+20	+14½
2R	60 mm	50 mm	10	100 mm	95 mm	+5	E		
3L	110 mm	90 mm	20	140 mm	110 mm	10	V	+5	+3½
3R	105 mm	90 mm	15	190 mm	180 mm	+5	E		
4L	80 mm	70 mm	10	110 mm	45 mm	55	E	55	+19
4R	65 mm	50 mm	15	80 mm	65 mm	0	V		
5L	70 mm	70 mm	0	90 mm	60 mm	30	E	5	+3½
5R	65 mm	55 mm	10	95 mm	60 mm	25	V		
6L	80 mm	70 mm	10	110 mm	95 mm	-5	V	15	+10
6R	60 mm	60 mm	0	70 mm	50 mm	20	E		
7L	60 mm	50 mm	10	95 mm	80 mm	5	E	5	+3½
7R	65 mm	60 mm	5	95 mm	90 mm	0	V		
8L	70 mm	70 mm	0	100 mm	100 mm	0	V	30	+17½
8R	50 mm	50 mm	0	100 mm	70 mm	30	E		
9L	100 mm	70 mm	30	105 mm	95 mm	-20	V	0	+1½
9R	80 mm	60 mm	20	110 mm	110 mm	-20	E		
10L	60 mm	50 mm	10	85 mm	75 mm	0	E	0	+1½
10R	50 mm	50 mm	0	90 mm	90 mm	0	V		
11L	40 mm	40 mm	0	110 mm	80 mm	30	E	20	+14
11R	40 mm	40 mm	0	95 mm	85 mm	10	V		
12L	40 mm	40 mm	0	65 mm	60 mm	5	V	15	+10
12R	50 mm	50 mm	0	80 mm	60 mm	20	E		
13L	40 mm	40 mm	0	60 mm	60 mm	0	V	15	+10
13R	40 mm	40 mm	0	110 mm	95 mm	15	E		

TABLE 4:1 (continued)

RAT	PREOPERATIVE P TD	PREOPERATIVE D TD	PREOPERATIVE DIFF	POSTOPERATIVE P DIA	POSTOPERATIVE D DIA	RESULT DIFF	RESULT SUTURE	ETHILON -VICRYL	SIGNED RANK
14L	50 mm	50 mm	0	110 mm	70 mm	40	V	-20	-14½
14R	80 mm	80 mm	0	110 mm	90 mm	20	E		
15L	50 mm	45 mm	5	125 mm	100 mm	20	E	15	+10
15R	40 mm	40 mm	0	50 mm	45 mm	5	V		
16L	55 mm	50 mm	5	110 mm	80 mm	25	E	15	+10
16R	50 mm	50 mm	0	100 mm	90 mm	10	V		
17L	50 mm	50 mm	0	115 mm	95 mm	20	V	-20	-14½
17R	55 mm	50 mm	5	110 mm	105 mm	0	E		
19L	60 mm	60 mm	0	50 mm	50 mm	0	V	30	+17½
19R	60 mm	60 mm	0	100 mm	70 mm	30	E		
20L	90 mm	90 mm	0	100 mm	85 mm	15	E	-5	-3½
20R	80 mm	80 mm	0	100 mm	80 mm	20	V		

Rat 18 unsuitable for assessment

Projected onto screen from 20 metres - size of slide 23 x 35 mm
size on screen 1200 x 1800 mm

T(-) = 39.5 for 19 pairs, ie Significant

T(+) = 146.5

Wilcoxon Signed Rank Sum Test is Significant

P = proximal, D = distal, TD = transverse diameter

Kendal's Coefficient of Rank Correlation

r = 0.045 Non significant

TABLE 4.2a - OBSERVER SCORE (2 OBSERVERS FROM 5 VIEWINGS EACH SCORED OUT OF 10) VS DIFFERENCE IN AXON COUNT (n = 17)

Correlation of Vicryl

NO	OBSERVERS (X)	LOG (Y)	X^2	Y^2	X x Y
1	39	0.197	152	0.039	7.68
2	45	0.134	2025	0.018	6.03
3	40	0.117	1600	0.014	4.68
4	4	0.191	16	0.036	0.764
5	21	0.035	441	0.0012	0.735
6	4	0.385	16	0.148	1.54
7	0	0.176	0	0.031	0
8	48	0.196	2304	0.038	9.41
9	60	0.586	3600	0.343	35.16
10	48	0.008	2304	0.00	0.38
11	42	0.04	1764	0.002	1.68
12	90	0.24	8100	0.58	21.6
13	90	0.356	8100	0.127	32.04
14	56	0.809	3136	0.654	45.3
15	30	0.065	900	0.004	1.95
16	48	0.042	2304	0.002	2.02
19	100	0.168	10000	0.028	16.8
	765	3.745	46762	2.065	187.77

Rat 17, 18 and 20 unsuitable for assessment

Wilcoxon Signed Rank Sum Test is non-significant

Kendal's Coefficient of Rank Correlation

P = 64 Q = 36 T = 5 u = 0

r = 0.2 Non significant

$$r = \frac{P - Q}{\sqrt{\left[\frac{1}{2} n (n-1) - T\right]\left[\frac{1}{2} n (n-1) - u\right]}}$$

TABLE 4.2b - OBSERVER SCORE (2 OBSERVERS FROM 5 VIEWINGS EACH SCORED OUT OF 10) VS DIFFERENCE IN AXON COUNT (n= 17)

Correlation of Ethilon

NO	OBSERVERS (X)	LOG (Y)	X^2	Y^2	X x Y
1	27	0.192	729	0.0369	5.184
2	60	1.004	3600	1.008	60.24
3	30	0.330	900	0.1089	9.9
4	7	0.631	49	0.398	4.417
5	60	0.167	3600	0.0279	10.02
6	5	0.049	25	0.002	0.245
7	60	0.028	3600	0.0001	1.68
8	40	0.278	1600	0.077	11.12
9	60	0.340	3600	0.116	20.4
10	33	0.204	1089	0.042	6.732
11	30	0.263	900	0.069	7.89
12	18	0.352	324	0.124	6.34
13	0	0.314	0	0.099	0
14	39	0.269	1521	0.0724	10.49
15	16	1.225	256	1.50	19.6
16	14	1.038	196	1.077	14.53
19	76	0.343	5776	0.1187	26.07
	614	7.027	27765	4.876	214.86
Mean	36.12	0.413			

Rat 17, 18 and 20 unsuitable for assessment

Wilcoxon Signed Rank Sum Test is non-significant

Kendal's Coefficient of Rank Correlation

P = 45 Q = 42 T = 4 u = 0

r = 0.02 Non significant

TABLE 4.2c - A CORRELATION OF OBSERVER VS DIFFERENCE IN AXON ACROSS THE ANASTOMOSIS.
VICRYL AND ETHILON SEPARATELY ASSESSED

RAT	OBSERVER FIGURE PHOTO	LOG OF PROXIMAL AND DISTAL AXON COUNT	SUTURE
1L	27	0.192	E
1R	39	0.197	V
2L	45	0.134	V
2R	60	1.004	E
3L	40	0.117	V
3R	30	0.330	E
4L	7	0.631	E
4R	4	0.191	V
5L	60	0.167	E
5R	21	0.035	V
6L	4	0.385	V
6R	5	0.049	E
7L	60	0.028	E
7R	0	0.176	V
8L	48	0.196	V
8R	40	0.278	E
9L	60	0.586	V
9R	60	0.340	E
10L	33	0.204	E
10R	48	0.008	V

TABLE 4.2c (continued)

RAT	OBSERVER FIGURE PHOTO	LOG OF PROXIMAL AND DISTAL AXON COUNT	SUTURE
11L	30	0.263	E
11R	42	0.040	V
12L	90	0.240	V
12R	18	0.352	E
13L	90	0.356	V
13R	0	0.314	E
14L	56	0.809	V
14R	39	0.269	E
15L	16	1.225	E
15R	30	0.065	V
16L	14	1.038	E
16R	48	0.042	V
17L	42	0.174	V
17R	14	0.096	E
18L	20		
18R	--		
19L	100	0.168	V
19R	76	0.343	E
20L	72		
20R	--		

Rat 18 and 20 unsuitable for assessment

Wilcoxon Signed Rank Sum Test is non-significant

Kendal's Coefficient of Rank Correlation

P = 175 Q = 161 T = 37 u = 0

r = 0.02 Non significant

TABLE 4.3 - MINIMAL EXCITABILITY TEST: A comparison of sutures with each other. Each nerve stimulated before division + at 10 weeks and the difference taken in volts.

RAT	ETHICON DIFFERENCES	VICRYL DIFFERENCES	ETHILON-VICRYL	RANK	SIGNED RANK
1	-1	6	-7	13	-13
2	37	19	18	16	+16
3	2	6	-4	9	-9
4	8	3	5	11½	+11½
5	5	5	0		
6	7	7	0		
7	-1	-1	0		
8	0	1	-1	2½	-2½
9	-1	-2	+1	2½	+2½
10	4	3	1	2½	+2½
11	1	9	-8	14	-14
12	4	1	3	6½	+6½
13	3	0	3	6½	+6½
14	-1	1	-2	5	-5
15	-3	2	-5	11½	-11½
16	7	3	4	9	+9
17	0	-1	+1	2½	+2½
19	1	10	-9	15	-15
20	10	6	4	9	+9

Rat 18 unsuitable for assessment T(-) 70
T(+) 60

Wilcoxon Signed Rank Sum Test is non-significant

Kendal's Coefficient of Rank Correlation

P = 83 Q = 31 T = 10 u = 10

r = 0.32 Non significant

TABLE 4.4 - MAXIMAL EXCITABILITY TEST: A comparison of Ethicon and Vicryl. Each nerve is stimulated before division and at 10 weeks postop and the difference taken in volts.

RAT	ETHICON DIFFERENCES	VICRYL DIFFERENCES	ETHILON-VICRYL	RANK	SIGNED RANK
1	0	-12	+12	13	+13
2	44	37	7	6½	+6½
3	-3	-17	+14	15	+15
4	-17	9	-26	17	-17
5	0	7	-7	6½	-6½
6	11	0	11	12	+12
7	-11	-6	-5	3½	-3½
8	-8	2	-10	10½	-10½
9	+2	15	-13	14	-14
10	18	10	8	9	+9
11	-3	29	-32	18	-18
12	-23	-8	-15	16	-16
13	-3	2	-5	3½	-3½
14	8	15	-7	6½	-6½
15	-22	-12	-10	10½	-10½
16	7	14	-7	6½	-6½
17	-7	-11	+4	2	+2
19	14	-19	33	19	+19
20	10	11	-1	1	-1

Rat 18 unsuitable for assessment T(-) = 113.5

T(+) = 76.5

Wilcoxon Signed Rank Sum Test is non-significant

Kendal's Coefficient of Rank Correlation

P = 92 Q = 52 T = 4 u = 2

r = 0.24 Non significant

TABLE 4.5a - AXON COUNTS: Percentage change across anastomosis expressed as a log and a strict comparison of Ethilon versus Vicryl.

RAT	PROXIMAL	DISTAL	LOG P	LOG D	DIFF	SUTURE	ETHILON -VICRYL
1L	1712	2667	3.234	3.426	0.192	E	-0.005
1R	2080	3277	3.318	3.515	0.197	V	
2L	2536	3453	3.404	3.538	0.134	V	+1.138
2R	2189	217	3.340	2.336	-1.004	E	
3L	3097	2367	3.491	3.374	-0.117	V	-0.447
3R	1688	3603	3.227	3.557	0.330	E	
4L	574	2453	2.759	3.390	0.631	E	0.822
4R	4853	3129	3.686	3.495	-0.191	V	
5L	2688	3941	3.429	3.556	0.167	E	0.132
5R	2468	2671	3.392	3.427	0.035	V	
6L	3338	1375	3.523	3.138	-0.385	V	-0.336
6R	2588	2312	3.413	3.364	-0.049	E	
7L	1901	2026	3.279	3.307	0.028	E	-0.204
7R	3134	2088	3.496	3.320	-0.176	V	
8L	2451	3850	3.389	3.585	0.196	V	0.474
8R	3505	1851	3.545	3.267	-0.278	E	
9L	1407	5426	3.148	3.734	0.586	V	-0.246
9R	1843	4032	3.266	3.606	0.340	E	
10L	2847	4555	3.454	3.658	0.204	E	0.196
10R	2527	2574	3.403	3.411	0.008	V	
11L	1425	2615	3.154	3.417	0.263	E	+0.303
11R	2998	2735	3.477	3.437	0.040	V	
12L	1532	2658	3.185	3.425	0.240	V	-0.112
12R	1528	3432	3.184	3.536	0.352	E	
13L	3072	1351	3.487	3.131	0.356	V	-0.670
13R	1190	2456	3.076	3.390	0.314	E	
14L	2107	327	3.324	2.515	0.809	V	-1.078
14R	1919	3562	3.283	3.552	0.269	E	

P = proximal

D = distal

TABLE 4:5a (Continued)

RAT	PROXIMAL	DISTAL	LOG P	LOG D	DIFF	SUTURE	ETHILON -VICRYL
15L	257	4312	2.410	3.635	1.225	E	+1.29
15R	1908	1645	3.281	3.216	0.065	V	
16L	252	2748	2.401	3.439	1.038	E	0.996
16R	1940	2137	3.288	3.330	0.042	V	
19L	3680	2498	3.566	3.398	0.168	V	-0.551
19R	1964	4329	3.293	3.636	0.343	E	
20L	1998	3325	3.301	3.522	0.221	E	0.294
20R	2140	1807	3.330	3.257	0.073	V	

Rat 17 and 18 unsuitable for assessment.
The nearest root the best, therefore, the difference is always + ve

For both Ethilon and Vicryl T(-) = 63 = NS
Wilcoxon Signed Rank Sum Test is non-significant T(+) = 108 = NS

Kendal's Coefficient of Rank Correlation for both Ethilon and Vicryl
P = 259 Q = 371 T = 0 u = 0 r = -0.17 Non significant

Wilcoxon signed Rank Sum Test for Ethilon only T(-) = 29
0.05 > p > 0.01 significantly more axons in distal T(+) = 142
portion compared with proximal portion

Kendal's Coefficient of Rank Correlation for Ethilon only
P = 78 Q = 75 r = 0.02 Non significant

Wilcoxon signed Rank Sum Test for Vicryl only T(-) 100
T(+) 71 Non significant

Kendal's Coefficient of Rank Correlation for Vicryl only
P = 67 Q = 85 r = 0.12 Non significant

TABLE 4.5b - ANALYSIS OF VARIANCE (ALL VALUES /10^6)

SOURCE	SUM OF SQUARES	DF	MEAN SQUARE	VARIANCE RATIO	
Difference between sutures	0.498	1	0.5	0.51	NS
Difference between proximal and distal	5.74	1	5.74	5.92	<0.025
Interaction	8.08	1	8.08	8.33	<0.01
Residual	66.02	68	0.97		
Total	80.33	71			

TABLE 4.6 - AREA OF AXONS: Percentage area of axons of total nerve proximal and distal.

NERVE	PERCENTAGE AREA PROXIMAL	PERCENTAGE AREA DISTAL	PERCENTAGE DIFFERENCE	SUTURE	ETHILON -VICRYL	RANK	SIGNED RANK
1L	26.29	18.00	8.29	E	-11.21	12	-12
1R	29.80	10.30	19.50	V			
2L	13.23	17.21	3.98	V	+5.42	9	+9
2R	16.14	6.74	9.40	E			
3L	52.57	16.30	36.27	V	-29.83	17	-17
3R	13.58	7.14	6.44	E			
4L	6.4	28.1	21.7	E	16.38	14	+14
4R	12.4	17.72	5.32	V			
5L	7.00	17.62	10.62	E	4.9	8	+8
5R	29.03	23.31	5.72	V			
6L	25.89	15.16	10.73	V	22.01	16	+16
6R	38.66	5.92	32.74	E			
7L	12.95	11.63	1.32	E	-15.1	13	-13
7R	25.16	8.74	16.42	V			
8L	8.37	7.58	0.79	V	0.28	1	+1
8R	10.68	11.75	1.07	E			
9L	13.10	6.5	6.6	V	1.2	3	+3
9R	4.27	12.07	7.80	E			
10L	12.72	6.85	5.37	E	-4.57	7	-7
10R	19.31	9.37	9.94	V			

TABLE 4.6 (Continued)

NERVE	PERCENTAGE AREA PROXIMAL	PERCENTAGE AREA DISTAL	PERCENTAGE DIFFERENCE	SUTURE	ETHILON -VICRYL	RANK	SIGNED RANK
11L	14.33	8.62	5.71	E	-38.03	18	-18
11R	51.67	7.93	43.74	V			
12L	9.83	9.35	0.50	V	21.05	15	+15
12R	45.93	24.38	21.55	E			
13L	10.85	10.69	0.18	V	0.78	2	+2
13R	8.80	7.84	0.96	E			
14L	8.27	1.70	6.57	V	11	11	+11
14R	25.57	8.00	17.57	E			
15L	10.44	18.98	8.54	E	6.98	10	+10
15R	18.59	20.15	1.56	V			
16L	14.56	19.35	4.79	E	3.79	6	+6
16R	11.56	12.56	1.00	V			
19L	21.18	12.68	8.50	V	-3.27	5	-5
19R	12.07	6.84	5.23	E			
20L	10.85	6.54	4.31	E	-2.82	4	-4
20R	4.30	11.43	7.13	V			

Rat 17 and 18 unsuitable for assessment

Wilcoxon Signed Rank Sum Test is non-significant T(-) = 76 = NS
T(+) = 95 = NS

Kendal's Coefficient of Rank Correlation
P = 79 Q = 74
r = 0.03 Non significant

TABLE 4.7 - AXON DENSITY IN AXONS PER SQUARE MICRON IN PERINEURIUM

	PROXIMAL			DISTAL			
NERVE	AXONS	AREA	DENSITY	AXONS	AREA	DENSITY	PERCENTAGE DIFFERENCE
1L	1712	95327	0.01796	2667	109419	0.02437	35.70 E
1R	2080	212251	0.00980	3277	114129	0.02871	192.96 V
2L	2536	134276	0.01890	3435	77274	0.04445	135.19 V
2R	2189	89291	0.02452	217	52760	0.00411	496.60 E
3L	3097	10236	0.30256	2367	161987	0.01461	1970.91 V
3R	1688	11520	0.14653	3603	90695	0.03973	269.96 E
4L	574	73904	0.007779	2453	64091	0.03827	392.54 E
4R	4853	147741	0.03285	3129	192647	0.01624	102.28 V
5L	2688	138071	0.01947	3941	117496	0.03354	72.27 E
5R	2468	149023	0.01656	2671	124743	0.02141	29.29 V
6L	3338	140761	0.02371	1375	140738	0.00977	142.68 V
6R	2588	246118	0.01052	2312	74221	0.03115	196.10 E
7L	1901	147112	0.01292	2026	71140	0.02848	120.43 E
7R	3134	121533	0.02579	2088	76921	0.02714	5.23 V
8L	2451	80387	0.03049	3850	87558	0.04400	44.30 V
8R	3505	141312	0.02480	1851	121690	0.01521	63.05 E
9L	1407	69180	0.02034	5426	156424	0.03469	70.55 V
9R	1843	68418	0.02694	4032	118697	0.03397	26.10 E
10L	2847	113657	0.02505	4555	100002	0.04555	81.84 E
10R	2527	190141	0.01329	2574	139375	0.01847	38.98 V

TABLE 4.7 (Continued)

	PROXIMAL			DISTAL				
NERVE	AXONS	AREA	DENSITY	AXONS	AREA	DENSITY	PERCENTAGE DIFFERENCE	
11L	1425	46258	0.03081	2615	111788	0.02340	31.17	E
11R	2998	673518	0.00445	2735	93951	0.02911	554.16	V
12L	1532	50073	0.03060	2658	58601	0.04536	48.24	V
12R	1528	96462	0.01584	3432	93743	0.03661	131.12	E
13L	3072	99550	0.03086	1351	118525	0.01140	170.70	V
13R	1190	78771	0.01511	2456	66516	0.03692	144.34	E
14L	2107	88381	0.02384	327	17069	0.01916	24.43	V
14R	1919	77558	0.02474	3562	112085	0.03178	28.46	E
15L	257	367000	0.00070	4312	137360	0.03139	3484.29	E
15R	1908	94935	0.02010	1645	103995	0.01582	27.05	V
16L	252	95234	0.00265	2748	109320	0.02514	848.68	E
16R	1940	80316	0.02415	2137	83542	0.02558	5.92	V
17L	3465	419572	0.00826	4058	99960	0.04060	391.53	V
17R	-	-	-	-	-	-	-	
18L	1205	745857	0.00162	3669	80572	0.04554	2711.11	E
18R	-	-	-	-	-	-	-	
19L	3680	138283	0.02661	2498	66273	0.03770	41.68	V
19R	1964	76275	0.02575	4329	115844	0.03737	45.13	E
20L	1998	90167	0.02216	3325	129329	0.02570	15.97	E
20R	2140	101115	0.02116	1807	48971	0.03690	74.39	V

Wilcoxon Signed Rank Sum Test is non-significant

Rat 17 and 18 were unsuitable for assessment

Kendal's Coefficient of Rank Correlation

P = 72 Q = 81

r = -0.07 Non significant

TABLE 5.5 - COMPARISON TRANSVERSE DIAMETER OF THE NERVE PROXIMAL AND DISTAL TO THE ANASTOMOSIS RELATIVE TO THE TRANSVERSE DIAMETER (FASCICULAR VS EPINEURIAL)

	ETHILON			
	PREOPERATIVE		POSTOPERATIVE	
RAT	PTD	DTD	PTD	DTD
1L	155	140	180	160
1R	200	180	190	190
2L	180	160	180	175
2R	175	190	190	160
3L	150	170	145	150
3R	160	160	175	165
4L	140	145	150	155
4R	100	105	120	110
5L	195	170	175	150
5R	180	180	190	180
11L	195	190	180	185
11R	180	185	190	160
12L	170	165	155	140
12R	150	160	180	175
13L	185	180	150	160
13R	160	170	190	190
14L	210	190	200	210
14R	190	185	185	190
15L	125	135	140	150
15R	145	140	135	145

Kendal's Coefficient of Rank Correlation

P = 10 Q = 13 T = 5 u = 6

r = 0.077 Non Significant

TABLE 5.5 (Continued)

VICRYL				
	PREOPERATIVE		POSTOPERATIVE	
RAT	PTD	DTD	PTD	DTD
6L	220	210	210	195
6R	210	210	200	200
7L	165	170	180	170
7R	100	120	120	140
8L	110	110	140	120
8R	100	115	120	105
9L	180	175	150	145
9R	160	150	150	130
10L	190	180	200	180
10R	195	195	190	195
16L	160	150	150	120
16R	175	180	180	170
17L	180	190	200	105
17R	190	175	150	165
18L	130	140	110	105
18R	145	130	150	170
19L	150	150	165	160
19R	180	175	170	190
20L	165	160	160	140
20R	175	170	150	130

Kendal's Coefficient of Rank Correlation

P = 17 Q = 22 T = 3 u = 5

r = 0.12 Non Significant

TABLE 5.6 - MINIMAL EXCITABILITY TEST

RAT	FASCICULAR DIFFS	EPINEURIAL DIFFS (ETHILON)
1	5	9
2	7	-4
3	-2	0
4	9	11
5	10	-5
11	0	6
12	5	8
13	0	0
14	20	14
15	-1	9

Kendal's Coefficient of Rank Correlation

P = 21 Q = 16 T = 2 u = 2

r = 0.12 Non significant

RAT	FASCICULAR DIFFS	EPINEURIAL DIFFS (VICRYL)
6	6	8
7	4	6
8	9	-2
9	2	5
10	13	21
16	1	-5
17	-6	10
18	9	2
19	12	3
20	18	4

Kendal's Coefficient of Rank Correlation

P = 23 Q = 17 T = 2 u = 0

r = 0.14 Non significant

TABLE 5.7 - MAXIMAL EXCITABILITY TEST

RAT	FASCICULAR DIFFS	EPINEURIAL DIFFS (ETHILON)
1.	22	16
2.	14	2
3.	8	9
4.	1	-9
5.	11	4
11.	12	6
12.	15	9
13.	0	-9
14.	-8	7
15.	9	2

Kendal's Coefficient of Rank Correlation

P = 30 Q = 12 T = 0 u = 3

r = 0.41 Non significant

RAT	FASCICULAR DIFFS	EPINEURIAL DIFFS (VICRYL)
6.	-6	11
7.	22	9
8.	1	19
9.	0	0
10.	4	1
16.	9	-2
17.	7	4
18.	-2	0
19.	13	0
20.	1	1

Kendal's Coefficient of Rank Correlation

P = 14 Q = 21 T = 1 u = 1

r = -0.16 Non significant

TABLE 6.5a - A COMPARISON OF THE TRANSVERSE DIAMETER OF THE NERVE PROXIMAL AND DISTAL TO THE ANASTOMOSIS RELATIVE TO THE TRANSVERSE DIAMETER PREOPERATIVELY

TUBE VS GLUE

	PREOPERATIVE		POSTOPERATIVE	
RAT	PTD	DTD	PTD	DTD
41L	75	75	70	70
41R	80	80	85	80
42L	80	80	80	80
42R	60	70	70	65
43L	70	60	75	70
43R	75	70	70	70
44L	90	85	90	85
44R	55	50	70	70
45L	40	40	75	60
45R	70	75	60	55
46L	55	50	50	50
46R	85	80	85	80
47L	85	75	90	90
47R	60	65	65	60
48L	65	65	60	60
48R	70	65	75	80
49L	45	40	50	80
49R	50	45	50	65
50L	50	45	70	50
50R	60	55	70	45

TABLE 6.5a (Continued)

TUBE VS GLUE

RAT	PREOPERATIVE PTD	PREOPERATIVE DTD	POSTOPERATIVE PTD	POSTOPERATIVE DTD
56L	80	70	75	75
56R	75	70	80	70
57L	80	80	80	75
57R	75	80	70	70
58L	40	45	50	50
58R	50	50	60	60
59L	80	80	75	75
59R	85	85	80	75
60L	55	50	50	50
60R	50	50	55	55
61L	65	65	80	75
61R	50	45	55	60
62L	55	50	55	65
62R	70	65	60	55
63L	60	60	70	55
63R	60	60	60	60
64L	80	75	85	80
64R	80	80	70	75
65L	70	75	75	70
65R	65	70	70	75

TABLE 6.5b - DIFFERENCE IN SIZE BEFORE AND 10 WEEKS AFTER ANASTOMOSIS

TUBE VS GLUE - NERVE CUT AND REPAIRED			
RAT	TUBE	RAT	GLUE
41L	0	41R	-5
42R	-15	42L	0
43L	+5	43R	+5
44R	+5	44L	0
45L	-15	45R	-10
46R	0	46L	+5
47L	+10	47R	-10
48R	+10	48L	0
49L	+30	49R	+20
50R	-20	50L	-15
56L	+10	56R	-5
57R	-5	57L	-5
58L	-5	58R	0
59R	-5	59L	0
60L	-5	60R	0
61R	+10	61L	-5
62L	+15	62R	0
63R	0	63L	-15
64L	0	64R	+5
65R	0	65L	+10

Kendal's Coefficient of Rank Correlation

P = 49 Q = 22 T = 24 u = 32

r = 0.167 Non Significant

TABLE 6.6 - MINIMAL EXCITABILITY TEST

RAT	TUBE DIFFS	GLUE DIFFS
41	-6	14
42	20	1
43	1	10
44	0	11
45	12	-15
46	11	2
47	2	3
48	18	15
49	25	-1
50	4	6
56	7	14
57	11	2
58	18	18
59	-6	1
60	2	10
61	0	6
62	-9	6
63	0	4
64	14	2
65	1	20

Kendal's Coefficient of Rank Correlation

P = 45 Q = 89 T = 8 u = 7

r = -0.24 Non significant

TABLE 6.7 - MAXIMAL EXCITABILITY TEST

RAT	TUBE DIFFS	GLUE DIFFS
41	8	8
42	-1	14
43	6	10
44	15	11
45	-7	2
46	2	3
47	-9	15
48	14	20
49	3	-12
50	-8	7
56	21	5
57	1	8
58	4	5
59	-15	6
60	9	11
61	-7	3
62	4	9
63	6	4
64	12	2
65	10	1

Kendal's Coefficient of Rank Correlation

P = 82 Q = 80 T = 3 u = 5

r = 0.01 Non significant